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ARTIFICIAL RECEPTORS INCLUDING REVERSIBLY IMMOBILIZED BUILDING BLOCKS, THE BUILDING BLOCKS, AND METHODS

Cross Reference to Related Applications

The present application is a continuation in part of U.S. Patent Application Serial No. 10/244,727, filed September 16, 2002, and of Application No. PCT/US03/05328, filed February 19, 2003, both entitled "ARTIFICIAL RECEPTORS, BUILDING BLOCKS, AND METHODS". The present application claims priority to the fullest extent to U.S. Provisional Patent Application Serial Nos. 60/459,062, filed March 28, 2003; 60/499,776, 60/499,867, 60/499,975, and 60/500,081, each filed September 3, 2003; and 60/526,511, filed December 2, 2003. The disclosures of each of these applications are incorporated herein by reference.

Introduction

The present invention relates to artificial receptors, to methods and compositions for making them, and to methods using them. A receptor provides a binding site for and binds a ligand. For example, at an elementary level, receptors are often visualized having a binding site represented as a lock or site into which a key or ligand fits. The binding site is lined with, for example, hydrophobic or functional groups that provide favorable interactions with the ligand.

The present invention provides compositions and methods for developing molecules that provide favorable interactions with a selected ligand. The present compositions and methods generate a wide variety of molecular structures, one or more of which interacts favorably with the selected ligand. Heterogeneous and immobilized combinations of building block molecules form the variety of molecular structures. For example, combinations of 2, 3, 4, or 5 distinct building block molecules immobilized near one another on a support provide molecular structures that serve as candidate and working artificial receptors. Figure 1 schematically illustrates an embodiment employing 4 distinct building blocks in a spot on a microarray to make a ligand binding site. This Figure illustrates a group of 4 building blocks at the corners of a square forming a unit cell. A group of four building blocks can be envisioned as the vertices on any quadrilateral. Figure 1 illustrates that spots or regions of building blocks can be envisioned as multiple unit cells, in this illustration

square unit cells. Groups of unit cells of four building blocks in the shape of other quadrilaterals can also be formed on a support.

The present artificial receptors include building blocks reversibly immobilized on a support or surface. Reversing immobilization of the building blocks can allow movement of building blocks to a different location on the support or surface, or exchange of building blocks onto and off of the surface.

For example, the combinations of building blocks can bind a ligand when reversibly coupled to or immobilized on the support. Reversing the coupling or immobilization of the building blocks provides opportunity for rearranging the building blocks, which can improve binding of the ligand. Further, the present invention can allow for adding additional or different building blocks, which can further improve binding of a ligand.

Figure 2 schematically illustrates an embodiment employing an initial artificial receptor surface (A) with four different building blocks on the surface, which are represented by shaded shapes. This initial artificial receptor surface (A) undergoes (1) binding of a ligand to an artificial receptor and (2) shuffling the building blocks on the receptor surface to yield a lead artificial receptor (B). Shuffling refers to reversing the coupling or immobilization of the building blocks and allowing their rearrangement on the receptor surface. After forming a lead artificial receptor, additional building blocks can be (3) exchanged onto and/or off of the receptor surface (C). Exchanging refers to building blocks leaving the surface and entering a solution contacting the surface and/or building blocks leaving a solution contacting the surface and becoming part of the artificial receptor. The additional building blocks can be selected for structural diversity (e.g., randomly) or selected based on the structure of the building blocks in the lead artificial receptor to provide additional avenues for improving binding. The original and additional building blocks can then be (4) shuffled and exchanged to provide higher affinity artificial receptors on the surface (D).

The present artificial receptors and methods can provide unique opportunities for discovering artificial receptors using high throughput screening strategies and then improving upon a lead artificial receptor discovered through the screening. In fact, embodiments of these compositions and methods can allow a lead receptor to improve itself. Although not limiting to the present invention, the reversibly immobilized building blocks can be

envisioned as providing equilibrium binding of a test ligand in a system in which the building blocks can be immobilized or mobile.

Background

The preparation of artificial receptors that bind ligands like proteins, peptides, carbohydrates, microbes, pollutants, pharmaceuticals, and the like with high sensitivity and specificity is an active area of research. None of the conventional approaches has been particularly successful; achieving only modest sensitivity and specificity mainly due to low binding affinity.

Antibodies, enzymes, and natural receptors generally have binding constants in the 10^8-10^{12} range, which results in both nanomolar sensitivity and targeted specificity. By contrast, conventional artificial receptors typically have binding constants of about 10^3 to 10^5, with the predictable result of millimolar sensitivity and limited specificity.

Several conventional approaches are being pursued in attempts to achieve highly sensitive and specific artificial receptors. These approaches include, for example, affinity isolation, molecular imprinting, and rational and/or combinatorial design and synthesis of synthetic or semi-synthetic receptors.

Such rational or combinatorial approaches have been limited by the relatively small number of receptors which are evaluated and/or by their reliance on a design strategy which focuses on only one building block, the homogeneous design strategy. Common combinatorial approaches form microarrays that include 10,000 or 100,000 distinct spots on a standard microscope slide. However, such conventional methods for combinatorial synthesis provide a single molecule per spot. Employing a single building block in each spot provides only a single possible receptor per spot. Synthesis of thousands of building blocks would be required to make thousands of possible receptors.

Conventional combinatorial methods provide practical access to only hundreds or thousands of different artificial receptors. The present inventor's Combinatorial Artificial Receptor Arrays™ (CARA™) can provide convenient access to one or 2 million different artificial receptors. Convenient access to more than a few million artificial receptors or candidates remains elusive.

There remains a need for practical methods providing access to significant numbers of artificial receptors. Thus, there remains a need for dynamic methods for making artificial receptors, for materials used in such dynamic methods, and for artificial receptors including reversibly immobilized building blocks.

Summary

The present invention relates to artificial receptors, arrays or microarrays of artificial receptors or candidate artificial receptors, and methods of making them. Each member of the array includes a plurality of building block compounds, which can be immobilized in a spot on a support. The present invention also includes the building blocks, combinations of building blocks, arrays of building blocks, and receptors constructed of these building blocks together with a support. The present invention also includes methods of using these arrays and receptors. The present invention also relates to artificial receptor including a plurality of building block compounds that are mobile or reversibly immobilized on a surface.

The present invention includes a method of making an array of artificial receptors including reversibly immobilized building blocks. This method includes forming a plurality of spots on a solid support. At least certain of the spots include a plurality of building blocks. The method includes reversibly immobilizing building blocks on the solid support in the spots.

The present invention includes a method of making a receptor surface or an artificial receptor. This method includes forming a region on a solid support. The region includes a plurality of building blocks. The method includes reversibly immobilizing building blocks on the solid support in the region.

The invention includes artificial receptors and compositions. The compositions can include a support and a plurality of building blocks. The compositions can also include a functionalized lawn. The functionalized lawn can be coupled to the support. Building blocks can be reversibly immobilized on the support, the lawn, or both. Reversible immobilization can employ any of a variety of reversible interactions, such as van der Waals, hydrophobic, or lipophilic interaction; a covalent bond; a hydrogen bond; an interaction between ions; or the like, or a combination thereof. The building blocks, the support, and or the functionalized lawn can include moieties that can form reversible immobilizing interactions,

such as hydrophobic interactions, a covalent bond, a hydrogen bond, an interaction between ions, or the like, or a combination thereof.

In an embodiment, the present invention includes a composition including a surface and a region on the surface. This region includes a plurality of building blocks, at least some of the building blocks being reversibly immobilized on the support.

The present invention includes arrays of artificial receptors and heterogeneous building block arrays. Such an array can include a support and a plurality of building blocks. The array can also include a functionalized lawn. The functionalized lawn can be coupled to the support. The array can also include a plurality of regions on the support. The regions can include a plurality of building blocks. Building blocks can be reversibly immobilized on the support, the lawn, or both.

The present invention includes kits and articles of manufacture. Such an article of manufacture can include a support and a plurality of building blocks. The article of manufacture can also include a functionalized lawn reagent. The functionalized lawn reagent can be configured to be coupled to the support. The plurality of building blocks can be configured to be reversibly coupled to the support, the lawn, or both.

The present invention includes methods of using an artificial receptor. These methods include shuffling building blocks and/or exchanging building blocks. In certain embodiments, shuffling can occur in or on one or more supports, surfaces, compositions, regions, spots or artificial receptors. In certain embodiments, exchanging building blocks can occur onto or off of one or more supports, surfaces, compositions, regions, spots or artificial receptors.

Brief Description of the Figures

Figure 1 schematically illustrates two dimensional representations of an embodiment of a receptor according to the present invention that employs 4 different building blocks to make a ligand binding site.

Figure 2 schematically illustrates an embodiment of the present methods and artificial receptors employing shuffling and exchanging building blocks

Figure 3A schematically illustrates representative structures of the support floor and building blocks according to the present invention on a surface of a support.

Figure 3B schematically illustrates a support coupled to a signal element, a building block, and a modified floor element.

Figure 4 schematically illustrates representative space filing structures of a candidate artificial receptor according to the present invention including both an amine floor and a four building block receptor.

Figure 5 schematically illustrates a glass support including pendant amine or amide structures.

Figure 6 schematically illustrates identification of a lead artificial receptor from among candidate artificial receptors.

Figure 7 schematically illustrates binding space divided qualitatively into 4 quadrants - large hydrophilic, large hydrophobic, small hydrophilic, and small lipophilic.

Figure 8 illustrates a plot of volume versus logP for 81 building blocks including each of the 9 A and 9 B recognition elements.

Figures 9A and 9B illustrate a plot of volume versus logP for combinations of building blocks with A and B recognition elements forming candidate artificial receptors. Figure 9B represents a detail from Figure 9A. This detail illustrates that the candidate artificial receptors fill the binding space evenly.

Figure 10 illustrates that candidate artificial receptors made up of building blocks can be sorted and evaluated with respect to their nearest neighbors, other candidate artificial receptors made up of one or more of the same building blocks.

Figure 11 schematically illustrates employing successive subsets of the available building blocks to develop a lead or working artificial receptor.

Figure 12 schematically illustrates positional isomers of combinations of 4 building blocks (A, B, C, and D) at vertices of a quadrilateral, and such isomers on a scaffold. The representations of the positional isomers on a scaffold include building blocks A, B, C, and D and a sphere representing a ligand of interest.

Figure 13A schematically illustrates an embodiment of an artificial receptor including building blocks reversibly immobilized through hydrophobic interactions with a lawn on a solid support. Figure 13B schematically illustrates that the building blocks can initially achieve a random distribution on a region of the support and then rearrange. This rearranging can form an improved or lead artificial receptor.

Figure 14 schematically illustrates an embodiment employing the present artificial receptors to develop a lead artificial receptor using shuffling and exchanging of building blocks.

Figure 15 schematically illustrates an embodiment of the artificial receptor shown in Figure 13A.

Figures 16A and 16B schematically illustrate embodiments of the artificial receptor shown in Figure 13A.

Figure 17 schematically illustrates test ligands with 3, 4, 5, 6, 7, or 8 binding surfaces or environments as polygons with 3, 4, 5, 6, 7, or 8 sides. A set of 81 building blocks in groups of 8 can provide up to about 32 billion candidate artificial receptors.

Figure 18 schematically illustrates serine as a framework for a building block and reactions for derivatizing the building block to add recognition elements.

Figure 19 schematically illustrates configurations in which recognition element(s), linker(s), and a chiral element can be coupled to a serine framework.

Figure 20 schematically illustrates embodiments of the present building blocks forming a candidate artificial receptor having a region suitable for binding a test ligand.

Figure 21 schematically illustrates embodiments of the present building blocks forming a candidate artificial receptor with a larger molecular footprint.

Figure 22 schematically illustrates embodiments of the present building blocks forming a candidate artificial receptor that is shown as suitable for binding a test ligand with a cavity.

Figure 23 schematically illustrates a false color fluorescence image of a labeled microarray according to an embodiment of the present invention.

Figure 24 schematically illustrates a two dimensional plot of data obtained for candidate artificial receptors contacted with and/or binding phycoerythrin.

Figure 25 schematically illustrates a three dimensional plot of data obtained for candidate artificial receptors contacted with and/or binding phycoerythrin.

Figure 26 schematically illustrates a two dimensional plot of data obtained for candidate artificial receptors contacted with and/or binding a fluorescent derivative of ovalbumin.

Figure 27 schematically illustrates a three dimensional plot of data obtained for candidate artificial receptors contacted with and/or binding a fluorescent derivative of ovalbumin.

Figure 28 schematically illustrates a two dimensional plot of data obtained for candidate artificial receptors contacted with and/or binding a fluorescent derivative of bovine serum albumin.

Figure 29 schematically illustrates a three dimensional plot of data obtained for candidate artificial receptors contacted with and/or binding a fluorescent derivative of bovine serum albumin.

Figure 30 schematically illustrates a two dimensional plot of data obtained for candidate artificial receptors contacted with and/or binding an acetylated horseradish peroxidase.

Figure 31 schematically illustrates a three dimensional plot of data obtained for candidate artificial receptors contacted with and/or binding an acetylated horseradish peroxidase.

Figure 32 schematically illustrates a two dimensional plot of data obtained for candidate artificial receptors contacted with and/or binding a TCDD derivative of horseradish peroxidase.

Figure 33 schematically illustrates a three dimensional plot of data obtained for candidate artificial receptors contacted with and/or binding a TCDD derivative of horseradish peroxidase.

Figure 34 schematically illustrates a subset of the data illustrated in Figure 25.

Figure 35 schematically illustrates a subset of the data illustrated in Figure 25.

Figure 36 schematically illustrates a subset of the data illustrated in Figure 25.

Figure 37 schematically illustrates a correlation of binding data for phycoerythrin against logP for the building blocks making up the artificial receptor.

Figure 38 schematically illustrates a correlation of binding data for phycoerythrin against logP for the building blocks making up the artificial receptor.

Figure 39 schematically illustrates a two dimensional plot comparing data obtained for candidate artificial receptors contacted with and/or binding phycoerythrin to data

obtained for candidate artificial receptors contacted with and/or binding a fluorescent derivative of bovine serum albumin.

Figures 40, 41, and 42 schematically illustrate subsets of data from Figures 25, 29, and 27, respectively, and demonstrate that the array of artificial receptors according to the present invention yields receptors distinguished between three analytes, phycoerythrin, bovine serum albumin, and ovalbumin.

Figure 43 schematically illustrates a gray scale image of the fluorescence signal from a scan of a control plate which was prepared by washing off the building blocks with organic solvent before incubation with the test ligand.

Figure 44 schematically illustrates a gray scale image of the fluorescence signal from a scan of an experimental plate which was incubated with 1.0 μg/ml Cholera Toxin B at 23 °C.

Figure 45 schematically illustrates a gray scale image of the fluorescence signal from a scan of an experimental plate which was incubated with 1.0 μg/ml Cholera Toxin B at 3 °C.

Figure 46 schematically illustrates a gray scale image of the fluorescence signal from a scan of an experimental plate which was incubated with 1.0 μg/ml Cholera Toxin B at 43 °C.

Figures 47-49 schematically illustrate plots of the fluorescence signals obtained from the candidate artificial receptors illustrated in Figures 44-46.

Figure 50 schematically illustrate plots of the fluorescence signals obtained from the combinations of building blocks employed in the present studies, when those building blocks are covalently linked to the support. Binding was conducted at 23 °C.

Figure 51 schematically illustrates a graph of the changes in fluorescence signal from individual combinations of building blocks at 4 °C, 23 °C, or 44 °C.

Detailed Description

Definitions

A combination of building blocks immobilized on, for example, a support can be a candidate artificial receptor, a lead artificial receptor, or a working artificial receptor. That is, a heterogeneous building block spot on a slide or a plurality of building blocks coated on a tube or well can be a candidate artificial receptor, a lead artificial receptor, or a working

artificial receptor. A candidate artificial receptor can become a lead artificial receptor, which can become a working artificial receptor.

As used herein the phrase "candidate artificial receptor" refers to an immobilized combination of building blocks that can be tested to determine whether or not a particular test ligand binds to that combination. In an embodiment, the combination includes one or more reversibly immobilized building blocks. In an embodiment, the candidate artificial receptor can be a heterogeneous building block spot on a slide or a plurality of building blocks coated on a tube or well.

As used herein the phrase "lead artificial receptor" refers to an immobilized combination of building blocks that binds a test ligand at a predetermined concentration of test ligand, for example at 10, 1, 0.1, or 0.01 μg/ml, or at 1, 0.1, or 0.01 ng/ml. In an embodiment, the combination includes one or more reversibly immobilized building blocks. In an embodiment, the lead artificial receptor can be a heterogeneous building block spot on a slide or a plurality of building blocks coated on a tube or well.

As used herein the phrase "working artificial receptor" refers to a combination of building blocks that binds a test ligand with a selectivity and/or sensitivity effective for categorizing or identifying the test ligand. That is, binding to that combination of building blocks describes the test ligand as belonging to a category of test ligands or as being a particular test ligand. A working artificial receptor can, for example, bind the ligand at a concentration of, for example, 100, 10, 1, 0.1, 0.01, or 0.001 ng/ml. In an embodiment, the combination includes one or more reversibly immobilized building blocks. In an embodiment, the working artificial receptor can be a heterogeneous building block spot on a slide or a plurality of building blocks coated on a tube, well, slide, or other support or on a scaffold.

As used herein the phrase "working artificial receptor complex" refers to a plurality of artificial receptors, each a combination of building blocks, that binds a test ligand with a pattern of selectivity and/or sensitivity effective for categorizing or identifying the test ligand. That is, binding to the several receptors of the complex describes the test ligand as belonging to a category of test ligands or as being a particular test ligand. The individual receptors in the complex can each bind the ligand at different concentrations or with different affinities. For example, the individual receptors in the complex each bind the ligand at

concentrations of 100, 10, 1, 0.1, 0.01 or 0.001 ng/ml. In an embodiment, the combination includes one or more reversibly immobilized building blocks. In an embodiment, the working artificial receptor complex can be a plurality of heterogeneous building block spots or regions on a slide; a plurality of wells, each coated with a different combination of building blocks; or a plurality of tubes, each coated with a different combination of building blocks.

As used herein, the term "building block" refers to a molecular component of an artificial receptor including portions that can be envisioned as or that include one or more linkers, one or more frameworks, and one or more recognition elements. In an embodiment, the building block includes a linker, a framework, and one or more recognition elements. In an embodiment, the linker includes a moiety suitable for reversibly immobilizing the building block, for example, on a support, surface or lawn. The building block interacts with the ligand.

As used herein, the term "linker" refers to a portion of or functional group on a building block that can be employed to or that does (e.g., reversibly) couple the building block to a support, for example, through covalent link, ionic interaction, electrostatic interaction, or hydrophobic interaction.

As used herein, the term "framework" refers to a portion of a building block including the linker or to which the linker is coupled and to which one or more recognition elements are coupled.

As used herein, the term "recognition element" refers to a portion of a building block coupled to the framework but not covalently coupled to the support. Although not limiting to the present invention, the recognition element can provide or form one or more groups, surfaces, or spaces for interacting with the ligand.

As used herein, the phrase "plurality of building blocks" refers to two or more building blocks of different structure in a mixture, in a kit, or on a support or scaffold. Each building block has a particular structure, and use of building blocks in the plural, or of a plurality of building blocks, refers to more than one of these particular structures. Building blocks or plurality of building blocks does not refer to a plurality of molecules each having the same structure.

As used herein, the phrase "combination of building blocks" refers to a plurality of building blocks that together are in a spot, region, or a candidate, lead, or working artificial receptor. A combination of building blocks can be a subset of a set of building blocks. For example, a combination of building blocks can be one of the possible combinations of 2, 3, 4, 5, or 6 building blocks from a set of N (e.g., N=10-200) building blocks.

As used herein, the phrases "homogenous immobilized building block" and "homogenous immobilized building blocks" refer to a support or spot having immobilized on or within it only a single building block.

As used herein, the phrase "activated building block" refers to a building block activated to make it ready to form a covalent bond to a functional group, for example, on a support. A building block including a carboxyl group can be converted to a building block including an activated ester group, which is an activated building block. An activated building block including an activated ester group can react, for example, with an amine to form a covalent bond.

As used herein, the term "naïve" used with respect to one or more building blocks refers to a building block that has not previously been determined or known to bind to a test ligand of interest. For example, the recognition element(s) on a naïve building block has not previously been determined or known to bind to a test ligand of interest. A building block that is or includes a known ligand (e.g., GM1) for a particular protein (test ligand) of interest (e.g., cholera toxin) is not naïve with respect to that protein (test ligand).

As used herein, the term "immobilized" used with respect to building blocks coupled to a support refers to building blocks being stably oriented on the support so that they do not migrate on the support or release from the support. Building blocks can be immobilized by covalent coupling, by ionic interactions, by electrostatic interactions, such as ion pairing, or by hydrophobic interactions, such as van der Waals interactions.

As used herein a "region" of a support, tube, well, or surface refers to a contiguous portion of the support, tube, well, or surface. Building blocks coupled to a region can refer to building blocks in proximity to one another in that region.

As used herein, a "bulky" group on a molecule is larger than a moiety including 7 or 8 carbon atoms.

As used herein, a "small" group on a molecule is hydrogen, methyl, or another group smaller than a moiety including 4 carbon atoms.

As used herein, the term "lawn" refers to a layer, spot, or region of functional groups on a support, for example, at a density sufficient to place coupled building blocks in proximity to one another. The functional groups can include groups capable of forming covalent, ionic, electrostatic, or hydrophobic interactions with building blocks.

As used herein, the term "alkyl" refers to saturated aliphatic groups, including straight-chain alkyl groups, branched-chain alkyl groups, cycloalkyl (alicyclic) groups, alkyl substituted cycloalkyl groups, and cycloalkyl substituted alkyl groups. In certain embodiments, a straight chain or branched chain alkyl has 30 or fewer carbon atoms in its backbone (e.g., C_1-C_{12} for straight chain, C_1-C_6 for branched chain). Likewise, cycloalkyls can have from 3-10 carbon atoms in their ring structure, for example, 5, 6 or 7 carbons in the ring structure.

The term "alkyl" as used herein refers to both "unsubstituted alkyls" and "substituted alkyls", the latter of which refers to alkyl moieties having substituents replacing a hydrogen on one or more carbons of the hydrocarbon backbone. Such substituents can include, for example, a halogen, a hydroxyl, a carbonyl (such as a carboxyl, an ester, a formyl, or a ketone), a thiocarbonyl (such as a thioester, a thioacetate, or a thioformate), an alkoxyl, a phosphoryl, a phosphonate, a phosphinate, an amino, an amido, an amidine, an imine, a cyano, a nitro, an azido, a sulfhydryl, an alkylthio, a sulfate, a sulfonate, a sulfamoyl, a sulfonamido, a sulfonyl, a heterocyclyl, an aryl alkyl, or an aromatic or heteroaromatic moiety. The moieties substituted on the hydrocarbon chain can themselves be substituted, if appropriate. For example, the substituents of a substituted alkyl can include substituted and unsubstituted forms of the groups listed above.

The phrase "aryl alkyl", as used herein, refers to an alkyl group substituted with an aryl group (e.g., an aromatic or heteroaromatic group).

As used herein, the terms "alkenyl" and "alkynyl" refer to unsaturated aliphatic groups analogous in length and optional substitution to the alkyls groups described above, but that contain at least one double or triple bond respectively.

The term "aryl" as used herein includes 5-, 6- and 7-membered single-ring aromatic groups that may include from zero to four heteroatoms, for example, benzene, pyrrole, furan,

thiophene, imidazole, oxazole, thiazole, triazole, pyrazole, pyridine, pyrazine, pyridazine and pyrimidine, and the like. Those aryl groups having heteroatoms in the ring structure may also be referred to as "aryl heterocycles" or "heteroaromatics". The aromatic ring can be substituted at one or more ring positions with such substituents such as those described above for alkyl groups. The term "aryl" also includes polycyclic ring systems having two or more cyclic rings in which two or more carbons are common to two adjoining rings (the rings are "fused rings") wherein at least one of the rings is aromatic, e.g., the other cyclic ring(s) can be cycloalkyls, cycloalkenyls, cycloalkynyls, aryls and/or heterocyclyls.

As used herein, the terms "heterocycle" or "heterocyclic group" refer to 3- to 12-membered ring structures, e.g., 3- to 7-membered rings, whose ring structures include one to four heteroatoms. Heterocyclyl groups include, for example, thiophene, thianthrene, furan, pyran, isobenzofuran, chromene, xanthene, phenoxathiin, pyrrole, imidazole, pyrazole, isothiazole, isoxazole, pyridine, pyrazine, pyrimidine, pyridazine, indolizine, isoindole, indole, indazole, purine, quinolizine, isoquinoline, quinoline, phthalazine, naphthyridine, quinoxaline, quinazoline, cinnoline, pteridine, carbazole, carboline, phenanthridine, acridine, pyrimidine, phenanthroline, phenazine, phenarsazine, phenothiazine, furazan, phenoxazine, pyrrolidine, oxolane, thiolane, oxazole, piperidine, piperazine, morpholine, lactones, lactams such as azetidinones and pyrrolidinones, sultams, sultones, and the like. The heterocyclic ring can be substituted at one or more positions with such substituents such as those described for alkyl groups.

As used herein, the term "heteroatom" as used herein means an atom of any element other than carbon or hydrogen, such as nitrogen, oxygen, sulfur and phosphorous.

Methods of Making an Artificial Receptor

Methods of Making Artificial Receptors Including Reversibly Immobilized Building Blocks

The present invention includes a method of producing an artificial receptor or a candidate artificial receptor. Producing an artificial receptor can include making an array of reversibly immobilized building blocks. Such a method can include forming a plurality of spots or regions on a support. At least some of the spots or regions in the array include a

plurality of building blocks. According to the present invention, the method includes reversibly immobilizing the plurality of building blocks on the support.

Reversibly immobilizing building blocks on a support couples the building blocks to the support through a mechanism that allows the building blocks to be uncoupled from the support without destroying or unacceptably degrading the building block or the support. That is, immobilization can be reversed without destroying or unacceptably degrading the building block or the support. In an embodiment, immobilization can be reversed with only negligible or ineffective levels of degradation of the building block or the support. Reversible immobilization can employ readily reversible covalent bonding or noncovalent interactions. Suitable noncovalent interactions include interactions between ions, hydrogen bonding, van der Waals interactions, and the like. Readily reversible covalent bonding refers to covalent bonds that can be formed and broken under conditions that do not destroy or unacceptably degrade the building block or the support.

In an embodiment, reversible immobilization of a building block employs a support functionalized to provide moieties on the support that can engage in a reversible interaction with the building block. In an embodiment, the support can be functionalized with moieties that can engage in reversible covalent bonding, moieties that can engage in noncovalent interactions, a mixture of these moieties, or the like.

The present invention can employ any of a variety of the numerous known functional groups, reagents, and reactions for forming reversible covalent bonds. Suitable reagents for forming reversible covalent bonds include those described in Green, TW; Wuts, PGM (1999), Protective Groups in Organic Synthesis Third Edition, Wiley-Interscience, New York, 779 pp. For example, the support can include functional groups such as a carbonyl group, a carboxyl group, a silane group, boric acid or ester, an amine group (e.g., a primary, secondary, or tertiary amine, a hydroxylamine, a hydrazine, or the like), a thiol group, an alcohol group (e.g., primary, secondary, or tertiary alcohol), a diol group (e.g., a 1,2 diol or a 1,3 diol), a phenol group, a catechol group, or the like. These functional groups can form groups with reversible covalent bonds, such as ether (e.g., alkyl ether, silyl ether, thioether, or the like), ester (e.g., alkyl ester, phenol ester, cyclic ester, thioester, or the like), acetal (e.g., cyclic acetal), ketal (e.g., cyclic ketal), silyl derivative (e.g., silyl ether), boronate (e.g.,

cyclic boronate), amide, hydrazide, imine, carbamate, or the like. Such a functional group can be referred to as a covalent bonding moiety, e.g., a first covalent bonding moiety.

A carbonyl group on the functionalized support and an amine group on a building block can form an imine or Schiff's base. The same is true of an amine group on the functionalized support and a carbonyl group on a building block. The imine or Schiff's base can be formed and cleaved under conditions that do not destroy or unacceptably degrade either the support or the building block.

A carbonyl group on the functionalized support and an alcohol group on a building block can form an acetal or ketal. The same is true of an alcohol group on the functionalized support and a carbonyl group on a building block. The acetal or ketal can be formed and cleaved under conditions that do not destroy or unacceptably degrade either the support or the building block.

A thiol (e.g., a first thiol) on the functionalized support and a thiol (e.g., a second thiol) on the building block can form a disulfide. The disulfide bond can be formed and cleaved under conditions that do not destroy or unacceptably degrade either the support or the building block.

A carboxyl group on the functionalized support and an alcohol group on a building block can form an ester. The same is true of an alcohol group on the functionalized support and a carboxyl group on a building block. Any of a variety of alcohols and carboxylic acids can form esters that provide covalent bonding that can be reversed in the context of the present invention. For example, readily reversible ester linkages can be formed from alcohols such as phenols with electron withdrawing groups on the aryl ring, other alcohols with electron withdrawing groups acting on the hydroxyl-bearing carbon, other alcohols, or the like; and/or carboxyl groups such as those with electron withdrawing groups acting on the acyl carbon (e.g., nitrobenzylic acid, R-CF_2-COOH, R-CCl_2-COOH, and the like), other carboxylic acids, or the like. Reversible ester linkages can be formed and cleaved under conditions that do not destroy or unacceptably degrade either the support, the lawn, or the building block.

In an embodiment, the support can be functionalized with moieties that can engage in noncovalent interactions. For example, the support can include functional groups such as an ionic group, a group that can hydrogen bond, or a group that can engage in van der Waals or

other hydrophobic interactions. Such functional groups can include cationic groups, anionic groups, lipophilic groups, amphiphilic groups, and the like. A cationic group on the functionalized support and an anionic group on a building block can form an ionic bond under conditions that do not destroy or unacceptably degrade either the support or the building block. The same is true of an anionic group on the functionalized support and a cationic group on a building block. By way of further example, an 18 carbon alkyl group on the functionalized support and a complementary lipophilic group on a building block can engage in a lipophilic interaction under conditions that do not destroy or unacceptably degrade either the support or the building block. The support can include a plurality of different moieties that can engage in assorted covalent or non-covalent interactions.

In an embodiment, the present methods and compositions can employ a support or substrate including a charged moiety (e.g., a first charged moiety). Suitable charged moieties include positively charged moieties and negatively charged moieties. Suitable positively charged moieties (e.g., at neutral pH in aqueous compositions) include amines, quaternary ammonium moieties, sulfonium, phosphonium, ferrocene, or the like. A positively charged moiety, such as a quaternary ammonium moiety, can also include one or more lipophilic moieties. Suitable negatively charged moieties (e.g., at neutral pH in aqueous compositions) include carboxylates, alkoxylates, phenols substituted with strongly electron withdrawing groups (e.g., tetrachlorophenols), phosphates, phosphonates, phosphinates, sulphates, sulphonates, thiocarboxylates, hydroxamic acids, or the like.

In an embodiment, the present methods and compositions can employ a support including groups that can hydrogen bond (e.g., a first hydrogen bonding group), either as donors or acceptors. The support can include a surface or region with groups that can hydrogen bond. For example, the support can include a surface or region including one or more carboxyl groups, amine groups, hydroxyl groups, carbonyl groups, or the like. Ionic groups can also participate in hydrogen bonding.

In an embodiment, the present methods and compositions can employ a support including a lipophilic moiety (e.g., a first lipophilic moiety). Suitable lipophilic moieties include branched or straight chain C_{6-36} alkyl, C_{8-24} alkyl, C_{12-24} alkyl, C_{12-18} alkyl, or the like; C_{6-36} alkenyl, C_{8-24} alkenyl, C_{12-24} alkenyl, C_{12-18} alkenyl, or the like, with, for example, 1 to 4 double bonds; C_{6-36} alkynyl, C_{8-24} alkynyl, C_{12-24} alkynyl, C_{12-18} alkynyl, or the like, with, for

example, 1 to 4 triple bonds; chains with 1-4 double or triple bonds; chains including aryl or substituted aryl moieties (e.g., phenyl or naphthyl moieties at the end or middle of a chain); polyaromatic hydrocarbon moieties; cycloalkane or substituted alkane moieties with numbers of carbons as described for chains; combinations or mixtures thereof; or the like. The alkyl, alkenyl, or alkynyl group can include branching; within chain functionality like an ether group; terminal functionality like alcohol, amide, carboxylate or the like; or the like. A lipophilic moiety like a quaternary ammonium lipophilic moiety can also include a positive charge. In an embodiment the lipophilic moiety includes or is a lipid, such as a phospholipid. In an embodiment, the lipophilic moiety includes or is a 16-carbon aliphatic moiety. In an embodiment, the lipid or support surface is in the form of or includes a lipid bilayer.

In an embodiment, reversible immobilization of a building block employs a support functionalized with a lawn reagent (e.g., a functionalized lawn reagent). The method can include coupling the lawn reagent to the support in, for example, a spot or region. The functionalized lawn reagent can provide functional groups that couple to the support plus moieties that engage in a reversible interaction with the building block. In an embodiment, the functionalized lawn reagent includes moieties that can engage in reversible covalent bonding, moieties that can engage in noncovalent interactions, mixtures of such moieties, or the like.

The functionalized lawn of the present invention can employ any of a variety of the numerous known functional groups, reagents, and reactions for forming reversible covalent bonds. Suitable reagents for forming reversible covalent bonds include those described in Green, TW; Wuts, PGM *supra*, and the others described above for supports. Such a functional group can be referred to as a covalent bonding moiety, e.g., a first covalent bonding moiety.

A carbonyl group on the functionalized lawn and an amine group on a building block can form an imine or Schiff's base. The same is true of an amine group on the functionalized lawn and a carbonyl group on a building block. The imine or Schiff's base can be formed and cleaved under conditions that do not destroy or unacceptably degrade either the support, the lawn, or the building block.

A carbonyl group on the functionalized lawn and an alcohol group on a building block can form an acetal or ketal. The same is true of an alcohol group on the functionalized

lawn and a carbonyl group on a building block. The acetal or ketal can be formed and cleaved under conditions that do not destroy or unacceptably degrade either the support, the lawn, or the building block.

A thiol (e.g., a first thiol) on the functionalized lawn and a thiol (e.g., a second thiol) on a building block can form an disulfide. The disulfide bond can be formed and cleaved under conditions that do not destroy or unacceptably degrade either the support, the lawn, or the building block.

A carboxyl group on the functionalized lawn and an alcohol group on a building block can form an ester. The same is true of an alcohol group on the functionalized lawn and a carboxyl group on a building block. Reversible ester linkages can be formed from alcohols and carboxyl groups described hereinabove. The reversible ester linkages can be formed and cleaved under conditions that do not destroy or unacceptably degrade either the support, the lawn, or the building block.

In an embodiment, the lawn reagent can be functionalized with moieties that can engage in noncovalent interactions. For example, the lawn reagent can include functional groups such as an ionic group, a group that can hydrogen bond, or a group that can engage in van der Waals or other hydrophobic interactions. Such functional groups can include cationic groups, anionic groups, lipophilic groups, amphiphilic groups, and the like. A cationic group on the functionalized lawn and an anionic group on a building block can form an ionic bond under conditions that do not destroy or unacceptably degrade either the support or the building block. The same is true of an anionic group on the functionalized support and a cationic group on a building block. By way of further example, an 18 carbon alkyl group on the functionalized lawn and a complementary lipophilic group on a building block can engage in a lipophilic interaction under conditions that do not destroy or unacceptably degrade either the support or the building block. The lawn can include a plurality of different moieties that can engage in assorted covalent or non-covalent interactions.

In an embodiment, the present methods and compositions can employ a lawn reagent including a charged moiety (e.g., a first charged moiety). Suitable charged moieties include positively charged moieties and negatively charged moieties. Suitable positively charged moieties include those described hereinabove. Suitable negatively charged moieties (e.g., at neutral pH in aqueous compositions) include those described hereinabove.

In an embodiment, the present methods and compositions can employ a building block including a charged moiety (e.g., a second charged moiety) that can interact with the lawn or support. Suitable charged moieties include those listed for lawn reagents.

In an embodiment, the present methods and compositions can employ a lawn reagent including a group that can hydrogen bond, either as donors or acceptors (e.g., a first hydrogen bonding group). Suitable hydrogen bonding groups include those described hereinabove. Ionic groups can also participate in hydrogen bonding.

In an embodiment, the present methods and compositions can employ a building block including a group that can hydrogen bond to the lawn or support (e.g., a second hydrogen bonding group). Suitable hydrogen bonding group include those listed for lawn reagents.

In an embodiment, the present methods and compositions can employ lawn reagent including a lipophilic moiety (e.g., a first lipophilic moiety). Suitable lipophilic moieties include those described hereinabove. In an embodiment, the lawn reagent includes a lipophilic moiety (e.g., a first lipophilic moiety) and a covalent bonding moiety (e.g., a first covalent bonding moiety). In an embodiment, the lawn reagent includes a lipophilic moiety (e.g., a first lipophilic moiety) and a charged moiety (e.g., a first charged moiety).

In an embodiment, the present methods and compositions can employ a building block including a lipophilic moiety (e.g., a second lipophilic moiety). Suitable lipophilic moieties include those described hereinabove. In an embodiment, the building block includes a lipophilic moiety (e.g., a second lipophilic moiety) and a covalent bonding moiety (e.g., a second covalent bonding moiety). In an embodiment, the building block includes a lipophilic moiety (e.g., a second lipophilic moiety) and a charged moiety (e.g., a second charged moiety).

In an embodiment, the lawn reagent includes a lipophilic moiety (e.g., a first lipophilic moiety) and a covalent bonding moiety (e.g., a first covalent bonding moiety) and the building block includes a lipophilic moiety (e.g., a second lipophilic moiety) and a covalent bonding moiety (e.g., a second covalent bonding moiety); the lawn reagent includes a lipophilic moiety (e.g., a first lipophilic moiety) and a charged moiety (e.g., a first charged moiety) and the building block includes a lipophilic moiety (e.g., a second lipophilic moiety) and a charged moiety (e.g., a second charged moiety); or combination thereof.

In an embodiment the present method of making an artificial receptor includes a method of making a receptor surface. Such a method can include forming a region on a solid support. The region can include a plurality of building blocks. The method can also include reversibly immobilizing the plurality of building blocks on the solid support in the region. In an embodiment, the present method of making an artificial receptor includes forming a region on a support that includes a plurality of building blocks. This embodiment can also include reversibly immobilizing the plurality of building blocks on the support in the region. The region can be a spot. These embodiments can include mixing the plurality of building blocks and employing the mixture in forming the plurality of spots, regions, or the receptor surface.

In an embodiment the present methods and compositions include reversibly and irreversibly coupled building blocks. For example, the present method can also include irreversibly coupling one or more building blocks to the support. In an embodiment, such an irreversibly coupled building block can be coupled through a covalent bond that cannot be broken without damaging the artificial receptor. In an embodiment, irreversible coupling employs a covalent bond that is stable under conditions used to reverse the reversible covalent bond. In an embodiment, an amide bond irreversibly couples a building block to a support.

Additional Methods of Making Artificial Receptors

The present invention relates to a method of making an artificial receptor or a candidate artificial receptor. In an embodiment, this method includes preparing a spot or region on a support, the spot or region including a plurality of building blocks immobilized on the support. The method can include forming a plurality of spots on a solid support, each spot including a plurality of building blocks, and coupling a plurality of building blocks to the solid support in each spot. In an embodiment, an array of such spots is referred to as a heterogeneous building block array.

The building blocks can be activated to react with a functional group on the support. Coupling can occur spontaneously after forming the spot of the building block or activated building block. The method can include mixing a plurality of activated building blocks and

employing the mixture in forming the spot(s). Alternatively, the method can include spotting individual activated building blocks on the support.

Forming a spot on a support can be accomplished by methods and apparatus such as pin spotters (sometimes referred to as printers), which can, for example, spot 10,000 to more than 100,000 spots on a microscope slide. Other spotters include piezoelectric spotters (similar to ink jets) and electromagnetic spotters that can also spot, for example, 10,000 to more than 100,000 spots on a microscope slide. An array of spots can also be printed on the bottom of a well of a microtiter plate. Arrays can also be built using photolithography and other known processes that can produce spots containing building blocks on a substrate. Conventional mixing valves or manifolds can be employed to mix the activated building blocks before spotting. These valves or manifolds can be under control of conventional microprocessor based controllers for selecting building blocks and amounts of reagents. Alternatively, the activated building blocks can be provided as mixtures made, for example, in large numbers in microwell plates by a robotic system.

Such spotting yields a microarray of spots of heterogeneous combinations of building blocks, each of which can be a candidate artificial receptor. Each spot in a microarray includes a statistically significant number of each building block. For example, although not limiting to the present invention, it is believed that each micro spot of a size sufficiently small that 100,000 fit on a microscope slide can include approximately 320 million clusters of 4 building blocks.

In an embodiment, the present method includes making a receptor surface. Making a receptor surface can include forming a region on a solid support, the region including a plurality of building blocks, and coupling the plurality of building blocks to the solid support in the region. The method can include mixing a plurality of activated building blocks and employing the mixture in forming the region or regions. Alternatively, the method can include applying individual activated building blocks in a region on the support. Forming a region on a support can be accomplished, for example, by soaking a portion of the support with the building block solution. A region including a plurality of building blocks can be independent and distinct from other regions including a plurality of building blocks. In an embodiment, one or more regions including a plurality of building blocks can overlap to produce a region including the combined pluralities of building blocks. In an embodiment,

two or more regions including a single building block can overlap to form one or more regions each including a plurality of building blocks. The overlapping regions can be envisioned, for example, as portions of overlap in a Ven diagram, or as portions of overlap in a pattern like a plaid or tweed.

In an embodiment, a tube or well coated with a support matrix can be filled with activated building block (e.g., a solution containing activated building block), which couples to the support matrix. For example, the support can be a glass tube or well coated with a plurality of building blocks. The surface of the glass tube or well can be coated with a coating to which the plurality of building blocks become covalently bound. The resulting coating including building blocks can be referred to as including heterogeneous building blocks.

In an embodiment, the method produces a surface or coating with a density of building blocks sufficient to provide interactions of more than one building block with a ligand. That is, the building blocks can be in proximity to one another. Proximity of different building blocks can be detected by determining different (e.g., greater) binding of a test ligand to a surface including a plurality of building blocks compared to a surface or surfaces including only one of the building blocks.

The method can apply or spot building blocks onto a support in combinations of 2, 3, 4, or more building blocks. For an embodiment employing a bulky tube or well, a manageable set of building blocks can provide fewer than several hundred or several thousand combinations of building blocks. For example, in this context, a set of 4, 5, or 6 building blocks provides a manageable number of combinations of 2, 3, or 4 building blocks. In an embodiment, the method can be employed to produce a plurality of tubes each tube having immobilized on its surface a heterogeneous combination of building blocks.

The method can apply or spot building blocks onto a support in combinations of 2 or 3 building blocks. Effective artificial receptors can be developed employing as few as several dozen or several hundred artificial receptors, that can include 2 and/or, preferably, 3 building blocks. Such artificial receptors can employ, for example, a tube, well, or slide as a support.

In an embodiment, the present method can be employed to produce a solid support having on its surface a plurality of regions or spots, each region or spot including a plurality

of building blocks. For example, the method can include spotting a glass slide with a plurality of spots, each spot including a plurality of building blocks. Such a spot can be referred to as including heterogeneous building blocks.

Each spot can include a density of building blocks sufficient to provide interactions of more than one building block with a ligand. Such interactions can be determined as described above for regions. The method can include spotting the building blocks so that each spot is separated from the others. A plurality of spots of building blocks is referred to herein as an array of spots.

In an embodiment, the method spots building blocks in combinations of 2, 3, 4, or more. The method can form up to 100,000 or more spots on a glass slide. Therefore, in this embodiment of the method, a manageable set of building blocks can provide several million combinations of building blocks. For example, in this context, a set of 81 building blocks provides a manageable number (1.66 million) of combinations of 4 building blocks. For convenience in limiting the number of slides employed in the method, in this embodiment a set includes up to 200 building blocks, e.g., 50-100, e.g., about 80 (including 81) building blocks.

In an embodiment, the method includes forming an array of heterogeneous spots made from combinations of a subset of the total building blocks and/or smaller groups of the building blocks in each spot. That is, the method forms spots including only, for example, 2 or 3 building blocks, rather than 4 or 5. For example, the method can form spots from combinations of a full set of building blocks (e.g. 81 of a set of 81) in groups of 2 and/or 3. For example, the method can form spots from combinations of a subset of the building blocks (e.g., 25 of the set of 81) in groups of 4 or 5. For example, the method can form spots from combinations of a subset of the building blocks (e.g., 25 of the set of 81) in groups of 2 or 3. The method can include forming additional arrays incorporating building blocks, lead artificial receptors, or structurally similar building blocks.

In an embodiment, the method includes forming an array including one or more spots that function as controls for validating or evaluating binding to artificial receptors of the present invention. In an embodiment, the method includes forming one or more regions, tubes, or wells that function as controls for validating or evaluating binding to artificial receptors of the present invention. Such a control spot, region, tube, or well can include no

building block, only a single building block, only functionalized lawn, or combinations thereof.

The method can couple building blocks to supports using known methods for activating compounds of the types employed as building blocks and for coupling them to supports. Covalent coupling can produce artificial receptors sufficiently durable to be used repeatedly over a period of months. The method can employ building blocks including activated esters and couple them to supports including amine functional groups. The method can include activating a carboxyl group on a building block by derivatizing to form the activated ester. By way of further example, the method can couple building blocks including amine functional groups to supports including carboxyl groups. Pairs of functional groups that can be employed on building blocks and supports according to the method include nucleophile/electrophile pairs, such as amine and carboxyl (or activated carboxyl), thiol and maleimide, alcohol and carboxyl (or activated carboxyl), mixtures thereof, and the like.

The support can include any functional group suitable for forming a covalent bond with a building block. The support or the building block can include a functional group such as alcohol, phenol, thiol, amine, carbonyl, or like group. The support or the building block can include a carboxyl, alcohol, phenol, thiol, amine, carbonyl, maleimide, or like group that can react with or be activated to react with the support or the building block. The support can include one or more of these groups. A plurality of building blocks can include a plurality of these groups.

The support or the building block can include a good leaving group bonded to, for example, an alkyl or aryl group. The leaving group being "good" enough to be displaced by the alcohol, phenol, thiol, amine, carbonyl, or like group on the support or the building block. Such a support or the building block can include a moiety represented by the formula: R-X, in which X is a leaving group such as halogen (e.g., -Cl, -Br, or -I), tosylate, mesylate, triflate, and R is alkyl, substituted alkyl, cycloalkyl, heterocyclic, substituted heterocyclic, aryl alkyl, aryl, heteroaryl, or heteroaryl alkyl. The support can include one or more of these groups. A plurality of building blocks can include a plurality of these groups.

The method can employ any of the variety of known supports employed in combinatorial or synthetic chemistry (e.g., a microscope slide, a bead, a resin, a gel, or the like). Suitable supports include functionalized glass, such as a functionalized slide or tube,

glass microscope slide, glass plate, glass coverslip, glass beads, microporous glass beads, microporous polymer beads (e.g. those sold under the tradename Stratospheres™), silica gel supports, and the like. Suitable supports with hydrophobic surfaces include micelles and reverse micelles.

The support can include a support matrix of a compound or mixture of compounds having functional groups suitable for coupling to a building block. The support matrix can be, for example, a coating on a microscope slide or functionalizing groups on a bead, gel, or resin. Known support matrices are commercially available and/or include linkers with functional groups that are coupled beads, gels, or resins. The support matrix functional groups can be pendant from the support in groups of one (e.g., as a lawn of amines, a lawn of another functional group, or a lawn of a mixture of functional groups) or in groups of, for example, 2, 3, 4, 5, 6, or 7. The groups of a plurality of functional groups pendant from the support can be visualized as or can be scaffold molecules pendant from the support.

The surface of the support can be visualized as including a floor and the building blocks (Figures 3A, 3B, and 4). As illustrated in Figure 3A, addition of building blocks to an amine lawn can proceed through reaction of the amines to form building block amides with some of the amines remaining on the floor of the support or candidate artificial receptor. Thus, the floor can be considered a feature of the candidate artificial receptor. The floor or modified floor can interact with the ligand as part of the artificial receptor. The nucleophilic or electrophilic groups on the floor can be left unreacted in the artificial receptor, or they can be modified. The floor can be modified with a small group that alters the recognition properties of the floor (Figure 3B). The floor can be modified with a signal element that produces a detectable signal when a test ligand is bound to the receptor (Figure 3B). For example, the signal element can be a fluorescent molecule that is quenched by binding to the artificial receptor. For example, the signal element can be a molecule that fluoresces only when binding occurs. The floor can be modified with a plurality of floor modifiers. For example, the floor can be modified with both a signal element and a small group that alters the recognition properties of the floor. One portion or region of the support can be modified with a first floor modifier or lawn and another (e.g., second) portion or region can be modified with a second floor modifier or lawn.

In an embodiment, the candidate artificial receptor can include building blocks and unmodified amines of the floor. Such a candidate artificial receptor has an amine/ammonium floor. In an embodiment, the candidate artificial receptor can include building blocks and modified amines of the floor. For example, the floor amines can be modified by the simplest amide modification of the amines to form the acetamide (e.g., by reacting with acetic anhydride or acetyl chloride). Alternatively, the floor amines can be modified by reaction with succinic anhydride, benzoyl chloride, or the like.

A lawn or other coating of functional groups can be derivatized with a maximum density of building blocks by exposing the lawn to several equivalents of activated building blocks. For example, less than 1 (e.g., 0.1) or more (e.g., 10) equivalents can be sufficient for an adequate density of building blocks on the support to observe building-block-dependent binding of a ligand. An amine modified glass surface can be functionalized with building blocks, for example, by reaction with activated carboxyl derivatives to form an amide link to the lawn.

For example, a building block linker carboxyl group can be activated by reacting the building block with carbodiimide in the presence of sulfo N-hydroxysuccinimide in aqueous dimethylformamide. The activated building block can be reacted directly with an amine on a glass support (hereinafter amino glass). Figure 3A illustrates that derivatization of only a portion of the amine groups on the support can be effective for producing candidate artificial receptors. Although not limiting to the present invention, it is believed that the amine load on the glass is in excess of that required for candidate artificial receptor preparation. Preparations of surfaces including combinations of building blocks can be accomplished by, for example, premixing of activated building blocks prior to addition to the amino tube or the sequential mixing of the coupling solutions in the tubes.

A commercially available glass support can be prepared for coupling building blocks by adding a support matrix to the surface of the support. The support matrix provides functional groups for coupling to the building block. Suitable support matrices include silanating agents. For example a glass tube (e.g., a 12x75 mm borosilicate glass tube from VWR) can be coated to form a lawn of amines by reaction of the glass with a silanating agent such as 3-aminopropyltriethoxysilane. Building blocks including an activated ester can be bound to this coating by reaction of the building block activated ester with the amine glass to

form the amide bound building block. Starting with a commercially available slide, an amino functionalized slide from Corning, building blocks including an activated ester can be spotted on and covalently bound to the slide in a micro array by this same reaction. Such derivatization is schematically illustrated in Figure 5.

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Using the Artificial Receptors

Using Artificial Receptors Including Reversibly Immobilized Building Blocks

The present invention includes a method of using artificial receptors. The present invention includes a method of screening candidate artificial receptors to find lead artificial
10 receptors that bind a particular test ligand. The method can then improve upon or test additional candidate or lead artificial receptors by allowing movement of the building blocks that make up the artificial receptors. Movement of building blocks can include mobilizing the building block to move along or on the support and/or to leave the support and enter a fluid (e.g., liquid) phase separate from the support or lawn.

15 In an embodiment, building blocks can be mobilized to move along or on the support (translate or shuffle). Such translation can be employed, for example, to allow building blocks already bound to a test ligand to rearrange into a lower energy or tighter binding configuration still bound to the test ligand. Such translation can be employed, for example, to allow the ligand access to building blocks that are on the support but not bound to the
20 ligand. These building blocks can translate into proximity with and bind to a test ligand.

Building blocks can be induced to move along or on the support or to be reversibly immobilized on the support through any of a variety of mechanisms. For example, inducing mobility of building blocks can include altering the conditions of the support or lawn. That is, altering the conditions can reverse the immobilization of the building blocks, thus
25 mobilizing them. Reversibly immobilizing the building blocks after they have moved can include, for example, returning to the previous conditions. Suitable alterations of conditions include changing pH, changing temperature, changing polarity or hydrophobicity, changing ionic strength, changing nucleophilicity or electrophilicity (e.g. of solvent or solute), and the like.

30 A variety of methods can be used to change the conditions of the surface or the building block. For example, fluid can be applied to the surface or lawn in an amount or of a

composition that can wet and/or change the conditions of the surface or lawn without providing bulk fluid into which building blocks can exchange. In an embodiment, the amount of fluid is sufficient to hydrate the surface or lawn without leaving any bulk solvent. In an embodiment, translation or shuffling can be achieved without exchanging by change in temperature, or the like.

A building block reversibly immobilized by hydrophobic interactions can be mobilized by increasing the temperature, by exposing the surface, lawn, or building block to a more hydrophobic solvent (e.g., an organic solvent or a surfactant), or by reducing ionic strength around the building block. In an embodiment, the organic solvent includes acetonitrile, acetic acid, an alcohol, tetrahydrofuran (THF), dimethylformamide (DMF), hydrocarbons such as hexane or octane, acetone, chloroform, methylene chloride, or the like, or mixture thereof. In an embodiment, the surfactant includes a nonionic surfactant, such as a nonylphenol ethoxylate, or the like. A building block that is mobile on a support can be reversibly immobilized by hydrophobic interactions, for example, by decreasing the temperature, exposing the surface, lawn, or building block to a more hydrophilic solvent (e.g., an aqueous solvent) or increased ionic strength.

A building block reversibly immobilized by hydrogen bonding can be mobilized by increasing the ionic strength, concentration of hydrophilic solvent, or concentration of a competing hydrogen bonder in the environs of the building block. A building block that is mobile on a support can be reversibly immobilized through an electrostatic interaction by decreasing ionic strength of the hydrophilic solvent, or the like.

A building block reversibly immobilized by an electrostatic interaction can be mobilized by increasing the ionic strength in the environs of the building block. Increasing ionic strength can disrupt electrostatic interactions. A building block that is mobile on a support can be reversibly immobilized through an electrostatic interaction by decreasing ionic strength.

A building block reversibly immobilized by an imine, acetal, or ketal bond can be mobilized by decreasing the pH or increasing concentration of a nucleophilic catalyst in the environs of the building block. In an embodiment, the pH is about 1 to about 4. Imines, acetals, and ketals undergo acid catalyzed hydrolysis. A building block that is mobile on a

support can be reversibly immobilized by a reversible covalent interaction, such as by forming an imine, acetal, or ketal bond, by increasing the pH.

In an embodiment, building blocks can be mobilized to leave the support and enter a fluid (e.g., liquid) phase separate from the support or lawn (exchange). For example, building blocks can be exchanged onto and/or off of the support. Exchange can be employed, for example, to allow building blocks on a support but not bound to a test ligand to be removed from the support. Exchange can be employed, for example, to add additional building blocks to the support. The added building blocks can have structures selected based on knowledge of the structures of the building blocks in artificial receptors that bind the test ligand. The added building blocks can have structures selected to provide additional structural diversity. The added building blocks can include all of the building blocks.

Building blocks can be induced to exchange on to and/or off of the support through any of a variety of mechanisms. For example, inducing exchange of building blocks can include contacting the building block with fluid. In an embodiment, contacting employs sufficient volume of the fluid to dilute the building block from the support. In an embodiment, contacting employs an amount and type of fluid that extracts the building block from the support. The contacting fluid can include reagents or have a characteristic that can reverse the immobilization of the building blocks, thus allowing them to exchange. In an embodiment, contacting employs a fluid containing a building block to be added to the support. The contacting fluid can include a reagent or have a characteristic that promotes reversible immobilization of the building blocks on the support.

For example, the fluid can have a pH, temperature, polarity or hydrophobicity, ionic strength, nucleophilicity or electrophilicity, and the like that promotes release of the building blocks from the support. Alternatively, the fluid can have a pH, temperature, polarity or hydrophobicity, ionic strength, nucleophilicity or electrophilicity, and the like that promotes reversible immobilization of the building blocks on the support.

A building block reversibly immobilized by hydrophobic interactions can be released from the support by, for example, raising the temperature, e.g., of the support and/or artificial receptor. For example, the hydrophobic interactions (e.g., the hydrophobic group on the support or lawn and on the building block) can be selected to provide immobilized building block at about room temperature or below and release can be accomplished at a temperature

above room temperature. For example, the hydrophobic interactions can be selected to provide immobilized building block at about refrigerator temperature (e.g., 4 °C) or below and release can be accomplished at a temperature of, for example, room temperature or above. By way of further example, a building block can be reversibly immobilized by hydrophobic interactions, for example, by contacting the surface or artificial receptor with a fluid containing the building block and that is at or below room temperature.

A building block reversibly immobilized by hydrophobic interactions can be released from the support by, for example, contacting the artificial receptor with a sufficiently hydrophobic fluid (e.g., an organic solvent or a surfactant). In an embodiment, the organic solvent includes acetonitrile, acetic acid, an alcohol, tetrahydrofuran (THF), dimethylformamide (DMF), hydrocarbons such as hexane or octane, acetone, chloroform, methylene chloride, or the like, or mixture thereof. In an embodiment, the surfactant includes a nonionic surfactant, such as a nonylphenol ethoxylate, or the like. Such reversible immobilization can also be effected by contacting the surface or artificial receptor with a hydrophilic solvent and allowing the somewhat lipophilic building block to partition on to the hydrophobic surface or lawn.

A building block reversibly immobilized by an imine, acetal, or ketal bond can be released from the support by, for example, contacting the artificial receptor with fluid having an acid pH or including a nucleophilic catalyst. In an embodiment, the pH is about 1 to about 4. A building block can be reversibly immobilized by a reversible covalent interaction, such as by forming an imine, acetal, or ketal bond, by contacting the surface or artificial receptor with fluid having a neutral or basic pH.

A building block reversibly immobilized by an electrostatic interaction can be released by, for example, contacting the artificial receptor with fluid having sufficiently high ionic strength to disrupt the electrostatic interaction. A building block can be reversibly immobilized through an electrostatic interaction by contacting the surface or artificial receptor with fluid having ionic strength that promotes electrostatic interaction between the building block and the support and/or lawn.

Embodiments Employing the Artificial Receptors Including Reversibly Immobilized Building Blocks

In an embodiment, the present invention includes a method of using an artificial receptor that includes translating or shuffling one or more building blocks in one or more regions on the support. Such a method can include contacting a reversibly immobilized heterogeneous molecular array with a test ligand and shuffling building blocks in one or more regions. This embodiment of the method can also include detecting binding of a test ligand to one or more regions and/or selecting one or more of the binding regions as the artificial receptor. The artificial receptor can be a lead artificial receptor. In this method, the building blocks in the array define a first set of building blocks, and the plurality of building blocks in the one or more binding regions defines one or more selected binding combinations of building blocks.

This embodiment of the method can employ an array including a support, a functionalized lawn, and a plurality of building blocks. The functionalized lawn can be coupled to the support. The array can include a plurality of regions on the support. The regions can include a plurality of building blocks. The plurality of building blocks can be reversibly immobilized on the lawn.

In an embodiment, the functionalized lawn includes a first covalent bonding moiety and the building block includes a second covalent bonding moiety. The first and second covalent bonding moieties form a readily reversible covalent bond. In this embodiment, shuffling includes contacting one or more regions to be shuffled with a composition including reagent promoting cleavage of the readily reversible covalent bond. In an embodiment, the reagent promoting cleavage has pH of about 1 to about 4.

In an embodiment, the functionalized lawn includes a first charged moiety and the building block includes a second charged moiety, the first and second charged moieties having opposite charges. In this embodiment, shuffling includes contacting one or more regions to be shuffled with a composition including reagent promoting separation of the first and second charged moieties. In an embodiment, the reagent includes salt concentration of about 0.1 to about 1 M.

In an embodiment, the functionalized lawn includes a first lipophilic moiety and the building block includes a second lipophilic moiety. In this embodiment, shuffling includes

contacting one or more regions to be shuffled with a composition including lipophilic reagent. In an embodiment, the lipophilic reagent includes organic solvent, surfactant, or mixture thereof. Suitable organic solvents and surfactants include those described hereinabove.

In an embodiment, the present invention includes a method of using an artificial receptor that includes exchanging one or more building blocks onto or off of one or more regions on the support. Such a method can include contacting a reversibly immobilized heterogeneous molecular array with a test ligand and exchanging one or more building blocks onto or off of the support. This embodiment of the method can also include detecting binding of a test ligand to one or more regions and/or selecting one or more of the binding regions as the artificial receptor. The artificial receptor can be a lead artificial receptor. In this method, the building blocks in the array define a first set of building blocks, and the plurality of building blocks in the one or more binding regions defines one or more selected binding combination of building blocks.

This embodiment of the method can employ an array including a support, a functionalized lawn, and a plurality of building blocks. The functionalized lawn can be coupled to the support. The array can include a plurality of regions on the support. The regions can include a plurality of building blocks. The plurality of building blocks can be reversibly immobilized on the lawn.

In an embodiment, exchanging includes contacting one or more regions with added building block and reversibly immobilizing the added building block in the region. In an embodiment, exchanging includes contacting one or more regions with reagent promoting release of reversibly immobilized building block and removing released building block. In an embodiment, exchanging includes contacting one or more regions with reagent promoting release of reversibly immobilized building block and removing released building block; and contacting one or more regions with added building block and reversibly immobilizing the added building block in the region.

In an embodiment, the functionalized lawn includes a first covalent bonding moiety and the building block includes a second covalent bonding moiety. The first and second covalent bonding moieties form a readily reversible covalent bond. In this embodiment, exchanging can include contacting one or more regions to be exchanged with an effective

volume of a fluid including reagent promoting cleavage of the readily reversible covalent bond. In this embodiment, exchanging can include contacting one or more regions to be exchanged with an effective volume of a fluid including one or more building blocks and reagent promoting formation of the readily reversible covalent bond.

In an embodiment, the functionalized lawn includes a first charged moiety and the building block includes a second charged moiety, the first and second charged moieties having opposite charges. In this embodiment, exchanging can include contacting one or more regions to be exchanged with a fluid including reagent promoting separation of the first and second charged moieties. In an embodiment, the reagent includes salt concentration of about 0.1 to about 2 M. In an embodiment, exchanging can include contacting one or more regions to be exchanged with a fluid including one or more building blocks and reagent promoting formation of ionic interactions.

In an embodiment, the functionalized lawn includes a first lipophilic moiety and the building block includes a second lipophilic moiety. In this embodiment, exchanging can include contacting one or more regions to be exchanged with a fluid including lipophilic reagent. In an embodiment, the lipophilic reagent includes organic solvent, surfactant, or mixture thereof. Suitable organic solvents and surfactants include those described hereinabove. In an embodiment, exchanging can include contacting one or more regions to be exchanged with a fluid including one or more building blocks and reagent promoting formation of hydrophobic interactions. In an embodiment, the reagent promoting formation of hydrophobic interactions includes water, or another nucleophilic or hydroxylic solvent.

In an embodiment, the method also includes determining the combinations of building blocks in one or more of the binding regions. The method can then include developing, based on the combinations determined, one or more developed sets of building blocks distinct from those in the one or more selected combinations of building blocks. This embodiment also includes exchanging into one or more of the regions one or more of the developed sets of building blocks. This embodiment can also include detecting binding of a test ligand to one or more of the exchanged regions and selecting one or more of the spots of the second heterogeneous molecular array as the artificial receptor. The artificial receptor can be a lead artificial receptor.

In an embodiment, this method includes varying the structure of the lead artificial receptor to increase binding speed or binding affinity of the test ligand. In an embodiment, the first set of building blocks includes a subset of a larger set of building blocks. In an embodiment, the first set of building blocks includes a subset of a larger set of building blocks, the second subset of building blocks defines a subset of the larger set of building blocks, and the first subset is not equivalent to the second subset. In an embodiment, the regions include 2, 3, or 4 building blocks.

In an embodiment, the method includes identifying the plurality of building blocks making up the artificial receptor; coupling the identified plurality of building blocks to a scaffold molecule; and evaluating the scaffold artificial receptor for binding of the test ligand. In an embodiment, coupling includes making a plurality of positional isomers of the building blocks on the scaffold; evaluating includes comparing the plurality of the scaffold positional isomer artificial receptors; and selecting one or more of the scaffold positional isomer artificial receptors as lead or working artificial receptor.

In an embodiment, the method includes applying the test ligand to one or more regions that function as controls for validating or evaluating binding to an artificial receptor. This embodiment can include employing a control region including no building block, only a single building block, only functionalized lawn, or a combination thereof.

Embodiments of methods including shuffling can also include exchanging building blocks onto or off of one or more regions. Embodiments of methods including exchanging can also include shuffling building blocks in one or more regions.

In an embodiment, the method includes shuffling before detecting. In an embodiment, the method includes detecting before shuffling. In an embodiment, the method includes shuffling, then detecting, then shuffling again. In an embodiment, the method includes contacting, then shuffling, then contacting again. In an embodiment, the method includes a combination thereof. In an embodiment, the method includes shuffling before detecting; detecting before shuffling; shuffling, then detecting, then shuffling again; contacting, then shuffling, then contacting again; or combinations thereof.

In an embodiment, this method includes shuffling before detecting. In an embodiment, the method includes detecting before shuffling. In an embodiment, the method includes shuffling, then detecting, then shuffling again. In an embodiment, the method

includes contacting, then shuffling, then contacting again. In an embodiment, the method includes exchanging before detecting. In an embodiment, the method includes detecting before exchanging. In an embodiment, the method includes exchanging, then detecting, then exchanging again. In an embodiment, the method includes contacting, then exchanging, then contacting again. In an embodiment, the method includes shuffling before exchanging. In an embodiment, the method includes exchanging before shuffling. In an embodiment, the method includes combinations thereof.

In an embodiment, the method includes shuffling before detecting; detecting before shuffling; shuffling, then detecting, then shuffling again; contacting, then shuffling, then contacting again; exchanging before detecting; detecting before exchanging; exchanging, then detecting, then exchanging again; contacting, then exchanging, then contacting again; shuffling before exchanging; exchanging before shuffling; or combinations thereof.

In an embodiment, the method includes shuffling before detecting. In an embodiment, the method includes detecting before shuffling. In an embodiment, the method includes shuffling, then detecting, then shuffling again. In an embodiment, the method includes contacting, then shuffling, then contacting again. In an embodiment, the method includes exchanging before detecting. In an embodiment, the method includes detecting before exchanging. In an embodiment, the method includes exchanging, then detecting, then exchanging again. In an embodiment, the method includes contacting, then exchanging, then contacting again. In an embodiment, the method includes shuffling before exchanging. In an embodiment, the method includes exchanging before shuffling. In an embodiment, the method includes combinations thereof.

In an embodiment, the method includes shuffling before detecting; detecting before shuffling; shuffling, then detecting, then shuffling again; contacting, then shuffling, then contacting again; exchanging before detecting; detecting before exchanging; exchanging, then detecting, then exchanging again; contacting, then exchanging, then contacting again; shuffling before exchanging; exchanging before shuffling; or combinations thereof.

In an embodiment, the method includes exchanging before detecting. In an embodiment, the method includes detecting before exchanging. In an embodiment, the method includes exchanging, then detecting, then exchanging again. In an embodiment, the method includes contacting, then exchanging, then contacting again. In an embodiment, the

method includes combinations thereof. In an embodiment, the method includes exchanging before detecting; detecting before exchanging; exchanging, then detecting, then exchanging again; contacting, then exchanging, then contacting again; or combinations thereof.

Detecting test ligand bound to a candidate artificial receptor can be accomplished using known methods for detecting binding to arrays on a slide or to coated tubes or wells. In an embodiment, the method employs test ligand labeled with a detectable label, such as a fluorophore or an enzyme that produces a detectable product. Alternatively, the method can employ an antibody (or other binding agent) specific for the test ligand and including a detectable label. The degree of labeling can be evaluated by evaluating the signal strength from the label. For example, the amount of signal can be directly proportional to the amount of label and binding.

According to the present method, screening candidate artificial receptors against a test ligand can yield one or more lead artificial receptors. One or more lead artificial receptors can be a working artificial receptor. That is, the one or more lead artificial receptors can be useful for detecting the ligand of interest as is. The method can then employ the one or more artificial receptors as a working artificial receptor for monitoring or detecting the test ligand. Alternatively, the one or more lead artificial receptors can be employed in the method for developing a working artificial receptor. For example, the one or more lead artificial receptors can provide structural or other information useful for designing or screening for an improved lead artificial receptor or a working artificial receptor. Such designing or screening can include making and testing additional candidate artificial receptors including combinations of a subset of building blocks, a different set of building blocks, or a different number of building blocks.

In certain embodiments, the method of the present invention can employ a smaller number of spots formed by combinations of a subset of the total building blocks and/or smaller groups of the building blocks. For example, the present method can employ an array including the number of spots formed by combinations of 81 building blocks in groups of 2 and/or 3. Then a smaller number of building blocks indicated by test compound binding, for example 36 building blocks, can be tested in a microarray with spots including larger groups, for example 4, of the building blocks.

Additional Methods of Using Artificial Receptors

The present invention includes a method of using artificial receptors. The present invention includes a method of screening candidate artificial receptors to find lead artificial receptors that bind a particular test ligand. Detecting test ligand bound to a candidate artificial receptor can be accomplished using known methods for detecting binding to arrays on a slide or to coated tubes or wells. For example, the method can employ test ligand labeled with a detectable label, such as a fluorophore or an enzyme that produces a detectable product. Alternatively, the method can employ an antibody (or other binding agent) specific for the test ligand and including a detectable label. One or more of the spots that are labeled by the test ligand or that are more or most intensely labeled with the test ligand are selected as lead artificial receptors. The degree of labeling can be evaluated by evaluating the signal strength from the label. The amount of signal can be directly proportional to the amount of label and binding. Figure 6 provides a schematic illustration of an embodiment of this process.

Binding to an array of candidate receptors can be displayed in any of a variety of graphs, charts, or illustrations. For example, a two dimensional array of candidate receptors can be displayed with signal strength as a third dimension. Such a representation of the array can be illustrated as a bar graph with the height of the bar from each spot in the array representing the signal strength. This representation can be useful, for example, for locating those candidate receptors in an array that show signal strength well in excess of other candidate receptors.

Candidate receptors can also be displayed in a chart correlating binding signal strength with one or more properties of the receptor and/or its constituent building blocks. For example, each candidate receptor can be located on a graph of the volume of its building blocks versus its lipophilicity/hydrophilicity (see, e.g., Figures 7-9B). Again, signal strength can be illustrated as a third dimension. Those candidate receptors showing the greatest binding can then be found and evaluated with respect to candidate receptors with similar properties (e.g., volume and lipophilicity/hydrophilicity).

Candidate receptors can also be displayed in a chart comparing binding signal strength with other candidate receptors including the same building blocks. For example, each candidate receptor can be located on a chart in which candidate receptors are grouped

by the building blocks that they contain (see, e.g., Figure 10). Again, signal strength can be illustrated as a third dimension. Those candidate receptors showing the greatest binding can then be found and evaluated with respect to candidate receptors including the same building blocks.

According to the present method, screening candidate artificial receptors against a test ligand can yield one or more lead artificial receptors. One or more lead artificial receptors can be a working artificial receptor. That is, the one or more lead artificial receptors can be useful for detecting the ligand of interest as is. The method can then employ the one or more artificial receptors as a working artificial receptor for monitoring or detecting the test ligand. Alternatively, the one or more lead artificial receptors can be employed in the method for developing a working artificial receptor. For example, the one or more lead artificial receptors can provide structural or other information useful for designing or screening for an improved lead artificial receptor or a working artificial receptor. Such designing or screening can include making and testing additional candidate artificial receptors including combinations of a subset of building blocks, a different set of building blocks, or a different number of building blocks.

In certain embodiments, the method of the present invention can employ a smaller number of spots formed by combinations of a subset of the total building blocks and/or smaller groups of the building blocks. For example, the present method can employ an array including the number of spots formed by combinations of 81 building blocks in groups of 2 and/or 3. Then a smaller number of building blocks indicated by test compound binding, for example 36 building blocks, can be tested in a microarray with spots including larger groups, for example 4, of the building blocks. Each set of microarrays can employ a different support matrix, lawn, or functionalized lawn. Such methods are schematically illustrated in Figure 11.

For example, Figure 11 illustrates that a single slide with the 3,240 combinations of 2 building blocks that can be produced from a set of 81 building blocks can be used to define a subset of the building blocks. This subset of, e.g., 25, building blocks (which can be derived from a 5x5 matrix of the results employing combinations of 2 building blocks), can be used to produce an additional 2,300 combinations of 3 building blocks and/or 12,650 combinations of 4 building blocks. These combinations from the subset can be screened to

define the optimum receptor configuration. The method can also include using combinations of building blocks in different ratios in spots.

On a macro scale, an artificial receptor presented as a spot or region including a plurality of building blocks has the plurality of building blocks distributed randomly throughout the spot or region. On a molecular scale, the distribution may not be random and even. For example, any selected group of only 2-10 building blocks may include a greater number of a particular building block or a particular arrangement of building blocks with respect to one another. A spot or region with a random distribution makes a useful artificial receptor according to the present invention. Particular assortments of building blocks found in a random distribution can also make useful artificial receptors.

An artificial receptor can include a particular assortment of a combination of 2, 3, 4, or more building blocks. Such an assortment can be visualized as occupying positions on the surface of a support. A combination of 2, 3, 4, or more building blocks can have each of the different building blocks in distinct positions relative to one another. For example, building block 1 can be adjacent to any of building blocks 2, 3, or 4. This can be illustrated by considering the building blocks at the vertices of a polygon. For example, Figure 12 illustrates positional isomers of 4 different building blocks at the vertices of a quadrilateral. By way of further example, 2 building blocks can be envisioned as located at points on a line, 3 building blocks can be envisioned as located at vertices of a triangle, 5 building blocks can be envisioned as located at the vertices of a pentagon, and so on.

In an embodiment of the method, a candidate artificial receptor can be optimized to a lead or working artificial receptor by making one or more of the positional isomers and determining its ability to bind the test ligand of interest. Advantageously, the positional isomers can be made on a scaffold (Figure 12). Scaffold positional isomer artificial receptors can be made, for example, on a scaffold with multiple functional groups that can be protected and deprotected by orthogonal chemistries. The scaffold positional isomer lead artificial receptors can be evaluated by any of a variety of methods suitable for evaluating binding of ligands to scaffold receptors. For example, the scaffold lead artificial receptors can be chromatographed against immobilized test ligand.

In an embodiment, the method of using an artificial receptor includes contacting a first heterogeneous molecular array with a test ligand. The array can include a support and a

plurality of spots of building blocks attached to the support. In the array, each spot of building blocks can include a plurality of building blocks with each building block being coupled to the support. The method includes detecting binding of a test ligand to one or more spots; and selecting one or more of the binding spots as the artificial receptor.

In this embodiment, the building blocks in the array can define a first set of building blocks, and the plurality of building blocks in each binding spot defines one or more selected binding combinations of building blocks. The first set of building blocks can include or be a subset of a larger set of building blocks. In an embodiment, the spots of building blocks can include 2, 3, or 4 building blocks. The first set can be immobilized using a first support matrix, a first lawn, or a first functionalized lawn.

In the method, the artificial receptor can include or be one or more lead artificial receptors. In the method, the artificial receptors can include or be one or more working artificial receptors.

This embodiment of the method can also include determining the combinations of building blocks in the one or more binding spots. These combinations can be used as the basis for developing one or more developed combinations of building blocks distinct from those in the one or more selected combinations of building blocks. This embodiment continues with contacting the test ligand with a second heterogeneous molecular array comprising a plurality of spots, each spot comprising a developed combination of building blocks; detecting binding of a test ligand to one or more spots of the second heterogeneous molecular array; and selecting one or more of the spots of the second heterogeneous molecular array as the artificial receptor. The second set can be immobilized using a second support matrix, a second lawn, or a second functionalized lawn different from those used with the first set.

In this embodiment, the building blocks in the second heterogeneous molecular array define a second set of building blocks. The first set of building blocks can include or be a subset of a larger set of building blocks and/or the second subset of building blocks can include or define a subset of the larger set of building blocks. Advantageously, the first subset is not equivalent to the second subset. In an embodiment, the spots of the second heterogeneous molecular array can include 3, 4, or 5 building blocks, and/or the spots of the

second heterogeneous molecular array can include more building blocks than the binding spots.

The artificial receptor can include or be a lead artificial receptor. The artificial receptor can include or be one or more working artificial receptors. The method can also include varying the structure of the lead artificial receptor to increase binding speed or binding affinity of the test ligand.

In an embodiment, the method includes identifying the plurality of building blocks making up the artificial receptor. The identified plurality of building blocks can then be coupled to a scaffold molecule to make a scaffold artificial receptor. This scaffold artificial receptor can be evaluated for binding of the test ligand. In an embodiment, coupling the identified plurality of building blocks to the scaffold can include making a plurality of positional isomers of the building blocks on the scaffold. Evaluating the scaffold artificial receptor can then include comparing the plurality of the scaffold positional isomer artificial receptors. In this embodiment, one or more of the scaffold positional isomer artificial receptors can be selected as one or more lead or working artificial receptors.

In an embodiment, the method includes screening a test ligand against an array including one or more spots that function as controls for validating or evaluating binding to artificial receptors of the present invention. In an embodiment, the method includes screening a test ligand against one or more regions, tubes, or wells that function as controls for validating or evaluating binding to artificial receptors of the present invention. Such a control spot, region, tube, or well can include no building block, only a single building block, only functionalized lawn, or combinations thereof.

Artificial Receptors

Artificial Receptors With Reversibly Immobilized Building Blocks

The present invention relates to artificial receptors and compositions that can form such receptors, e.g., candidate artificial receptors. The artificial receptors or compositions include building blocks reversibly immobilized on a support. The building blocks can be reversibly immobilized through any of a variety of interactions, such as covalent, electrostatic, or hydrophobic interactions.

In an embodiment, the composition includes molecules forming a lawn and coupled to the support. The building blocks can be reversibly immobilized through interactions with the lawn. In an embodiment, the present composition includes a support, a functionalized lawn, and a plurality of building blocks. The functionalized lawn can be coupled to the support. The plurality of building blocks can be reversibly immobilized on the lawn.

The building blocks can be reversibly immobilized on the lawn or support through, for example, readily reversible covalent bonding or noncovalent interactions. For such interactions, the lawn or support includes a functional group or moiety suitable for forming a readily reversible covalent bond or noncovalent interaction with the building block. Similarly, the building block includes a functional group or moiety suitable for forming a readily reversible covalent bond or noncovalent interaction with the lawn or support. For example, the building block and support or lawn each include one or more functional groups or moieties that can form readily reversible covalent, ionic, hydrogen bonding, van der Waals, or like interactions.

In an embodiment, the support includes a surface or region functionalized to include moieties suitable for a reversible interaction with the building block. In an embodiment, the support includes moieties that can engage in reversible covalent bonding or noncovalent interactions.

In an embodiment, the support includes moieties that can engage in reversible covalent bonding. Suitable groups for reversible covalent bonding are described hereinabove. A composition, such as a candidate artificial receptor, can include building blocks reversibly immobilized on the support through, for example, imine, acetal, ketal, disulfide, ester, and like linkages. An artificial receptor can include functional groups on the support that are not linked to a building block and support functional groups covalently linked to a building block.

In an embodiment, the support includes moieties that can engage in noncovalent interactions. For example, the support can include functional groups such as an ionic group, a group that can hydrogen bond, or a group that can engage in van der Waals or other hydrophobic interactions. A composition, such as a candidate artificial receptor, can include building blocks reversibly immobilized on the support through electrostatic interactions. An artificial receptor can include both free ionic groups on the support and support ionic groups

electrostatically linked to a building block. A composition, such as a candidate artificial receptor, can include building blocks reversibly immobilized on the support through hydrogen bonding. An artificial receptor can include both free hydrogen bonding groups on the support and support hydrogen bonding groups hydrogen bonded to a building block. A composition, such as a candidate artificial receptor, can include building blocks reversibly immobilized on the support through hydrophobic interactions. An artificial receptor can include both free hydrophobic groups on the support and support hydrophobic groups interacting with a building block.

In an embodiment, the support includes ionic groups, such as cationic groups, anionic groups, or mixtures thereof. The support can include a surface or region with ionic groups. For example, the support can include a surface or region including one or more cationic groups (e.g., at neutral pH in aqueous compositions) such as amines, quaternary ammonium moieties, sulfonium, phosphonium, ferrocene, or the like. For example, the support can include a surface or region including one or more anionic groups (e.g., at neutral pH in aqueous compositions) such as carboxylates, phenols substituted with strongly electron withdrawing groups (e.g., tetrachlorophenols), phosphates, phosphonates, phosphinates, sulphates, sulphonates, thiocarboxylates, hydroxamic acids, or the like. In an embodiment, the charge on the group relates to the charge at neutral pH in aqueous compositions.

In an embodiment, the support includes groups that can hydrogen bond, either as donors or acceptors. The support can include a surface or region with groups that can hydrogen bond. Suitable groups for hydrogen bonding include those described hereinabove. Ionic groups can also participate in hydrogen bonding.

In an embodiment, the support includes a hydrophobic or lipophilic group. The support can include a surface or region with hydrophobic or lipophilic groups. For example, the support can include a surface or region including one or more of the hydrophobic or lipophilic groups described hereinabove.

In an embodiment, the composition or artificial receptor includes a lawn (e.g., a functionalized lawn) coupled to a surface or region on the support. The lawn can be coupled to the support through covalent bonds that are stable under a variety of conditions such that it is difficult to remove the lawn from the support. For example, in an embodiment, the lawn cannot be uncoupled from the support under conditions that cleave a readily reversible

covalent bond. The lawn reagent can include any of a variety of functional groups that can be coupled to the support plus any of a variety of functional groups that can reversibly interact with the building block. For example, the lawn can include one or more moieties that can engage in reversible covalent bonding or noncovalent interactions with the building block.

In an embodiment, the lawn includes moieties that can engage in reversible covalent bonding. Suitable functional groups for reversible covalent bonding are described hereinabove. An artificial receptor can include building blocks reversibly immobilized on the lawn through imine, acetal, ketal, disulfide, ester, or like linkages. An artificial receptor can include both free functional groups on the lawn and lawn functional groups covalently linked to a building block.

In an embodiment, the lawn includes moieties that can engage in noncovalent interactions. For example, the lawn can include functional groups such as an ionic group, a group that can hydrogen bond, or a group that can engage in van der Waals or other hydrophobic interactions. An artificial receptor can include building blocks reversibly immobilized on the lawn through electrostatic interactions. Suitable functional groups for electrostatic interactions are described hereinabove. An artificial receptor can include both free ionic groups on the lawn and lawn ionic groups electrostatically linked to a building block.

An artificial receptor can include building blocks reversibly immobilized on the lawn through hydrogen bonding. Suitable functional groups for hydrogen bonding interactions are described hereinabove. An artificial receptor can include both free hydrogen bonding groups on the lawn and lawn hydrogen bonding groups hydrogen bonded to a building block.

An artificial receptor can include building blocks reversibly immobilized on the lawn through hydrophobic interactions. Suitable functional groups for hydrophobic interactions are described hereinabove. An artificial receptor can include both free hydrophobic groups on the lawn and lawn hydrophobic groups interacting with a building block.

In an embodiment the present methods and compositions can include building blocks that are coupled to the support in a manner that is essentially irreversible. For example, an irreversibly coupled building block can be coupled through a covalent bond that cannot be broken without damaging the artificial receptor. In an embodiment, irreversible coupling

employs a covalent bond that is stable under conditions used to reverse the reversible covalent bond. In an embodiment, an amide bond irreversibly couples a building block to a support. According to the present invention, an artificial receptor including n building blocks can include as many as n-1 irreversibly immobilized building blocks and 1 reversibly immobilized building block.

Illustrated Embodiments of Artificial Receptors

Figure 13A schematically illustrates an embodiment of an artificial receptor including building blocks reversibly immobilized through hydrophobic interactions with a lawn on a solid support. In this embodiment, the hydrophobic interactions are provided by long unbranched alkyl chains. Building blocks can be synthesized with long chain alkyl or alkyl-like linkers appended to the framework through, e.g., a carboxyl moiety. The support can include an amino surface modified by reaction with, e.g., activated long chain fatty acids to form an alkyl (or alkyl-like) lawn. Addition of the building blocks to the surface environment leads to incorporation of at least some of the building blocks into the lawn with the portion of the building block including the recognition elements (e.g., ligand binding portion) on the surface of the lawn. The surface of the artificial receptor can also include any of a variety of solvent environments.

Figure 13B schematically illustrates that the building blocks can achieve a random distribution on a region of the support and rearrange. Upon exposure to a test ligand, mobilized building blocks can rearrange to provide improved binding of the test ligand. Although not limiting the present invention, this binding and rearrangement can be envisioned as initial binding of a test ligand followed by kinetically and/or thermodynamically driven spatial redistribution of the building blocks. Such spatial redistribution can improve or optimize interactions between the artificial receptor and the test ligand. Such kinetic or thermodynamic improvement or optimization can be viewed as "evolution" toward greater binding affinity in an environment that can have mobile and/or immobilized building blocks.

Figure 14 schematically illustrates an embodiment employing the present artificial receptors to develop a lead artificial receptor using shuffling and exchanging of building blocks. View A of the artificial receptor schematically illustrates the building blocks in a

random distribution on a region of the support. The building blocks and lawn can include, for example, the alkyl tails schematically illustrated in Figure 13A.

Reaction 1 includes contacting the artificial receptor with a test ligand. Reaction 1 as illustrated also includes a change in temperature to allow building blocks to shuffle or rearrange within the receptor, which can improve binding to the test ligand. In another embodiment, shuffling or rearranging can be induced by other changes in conditions, such as change in solvent composition or a combination of change in temperature and solvent. View B of the artificial receptor schematically illustrates the rearranged building blocks with bound test ligand and also building blocks not bound to the test ligand.

Reaction 2 further mobilizes the building blocks and allows unbound building blocks to exchange off of the artificial receptor surface. Reaction 2 as illustrated also includes a change in temperature sufficient to allow building blocks to exchange into fluid contacting the artificial receptor. Such a temperature change may be larger than the temperature change in reaction 1. In another embodiment, this exchange can be accomplished, for example, by a change in conditions such as changing solvent composition (e.g., contacting with a more hydrophobic solvent), by increasing temperature and changing solvent composition, or the like. The change in conditions used to achieve exchange can be larger or more pronounced than a change used to achieve shuffling. Although not limiting to the present invention, this exchange can be viewed as increasing the on/off rate of the building blocks and leading to loss of the building blocks which are not protected by interaction with the target. View C of the artificial receptor schematically illustrates the rearranged building blocks with bound test ligand and the absence of building blocks exchanged off of the artificial receptor.

Reaction 3 exchanges additional building blocks onto the artificial receptor. Reaction 3 can include changing the conditions as described for exchanging building blocks off of the artificial receptor. View D schematically illustrates the artificial receptor including the added building blocks. Although not limiting to the present invention, the reaction can be considered affinity maturation of a receptor, exchanging one or more of the first set of building blocks for one or more building blocks which may have higher affinity for the test ligand.

Reaction 4, similar to reactions 1 and 2, shuffles or rearranges building blocks within the receptor and exchanges unbound building blocks off of the artificial receptor. This

reaction can use conditions as described for reactions 1 and 2. View E schematically illustrates the artificial receptor with shuffled and exchanged building blocks bound to the test ligand. In an embodiment, the artificial receptor with the added building blocks has greater affinity for the test ligand than did the preceding receptor-ligand complexes. Although not limiting to the present invention, this process can be considered as equilibrium driven affinity maturation.

Figure 15 schematically illustrates an embodiment of the artificial receptor shown in Figure 13A. This embodiment includes building blocks reversibly immobilized through hydrophobic interactions with a lawn on a solid support. The hydrophobic interactions are provided by long alkyl chains. The hydrophobic interactions by themselves can be sufficient to reversibly immobilize the building block. In addition, the lawn or support and the alkyl chain on the building block each include a functional group or moiety that can form a reversible covalent bond. Forming the covalent bond can fix the building block in a particular location on the support of the artificial receptor. The building block can, for example, remain fixed under conditions suitable to mobilize building blocks reversibly immobilized only by hydrophobic interactions. Such as system can provide selective mobility of some but not all building blocks. Breaking the covalent bond can allow the building block mobility within the hydrophobic environment of the artificial receptor (e.g., to translate or shuffle) and to be released from the support and hydrophobic environment (e.g., to exchange).

In this embodiment, the receptor can begin either with the building block fixed by the covalent bond, fixed by the hydrophobic interaction, or both. For example, the building blocks can be initially fixed in position by the reversible covalent bond. Breaking the reversible covalent bond can allow mobility of the building block. Mobilization can allow affinity optimization or improvement of the artificial receptor. Although not limiting to the present invention, this approach can allow greater initial time for kinetic and thermodynamic equilibration of interactions between the test ligand and the artificial receptor before the onset of more stringent conditions. By way of further example, the building blocks can initially be reversibly immobilized on or in a place on the lawn by hydrophobic interactions and then be fixed into position by a covalent bond after binding of a test ligand. Although not limiting to the present invention, this approach can allow fixing the artificial receptors in

a configuration useful for or optimal for binding test ligand, which can increase stability of the receptor:ligand complex.

Figures 16A and 16B schematically illustrate embodiments of the artificial receptor shown in Figure 13A. These embodiments include building blocks reversibly immobilized through hydrophobic interactions with a lawn on a solid support. The hydrophobic interactions are provided by alkyl chains on the support and/or building block. The lawn and/or the alkyl chain on the building block can each include one or more functional groups or moieties that can form a reversible bond, such as a reversible covalent bond, an ionic interaction, or a hydrogen bond. Figure 16A illustrates an embodiment in which building blocks can be reversibly bound one to the other. Figure 16B illustrates an embodiment in which one or more building blocks can be reversibly bound to one or more molecules making up the lawn. In the embodiments illustrated in Figures 16A and 16B, reversible bonds between the alkyl chains can control the position and/or mobility of the building blocks during or after binding of a test ligand. The various types of reversible immobilization of the present invention can provide variable degrees of building block mobility on the support.

Referring now to Figure 17, in an embodiment, a strategy employing the present artificial receptors with reversibly immobilized building blocks can provide convenient access to millions and even billions of different artificial receptors. Starting with, for example, 81 different building blocks, combinations of 2, 3, 4, 5, or more building blocks quickly yield more than several million artificial receptors including more than one building block. For example, 81 building blocks provide 85,320 combinations of three building blocks and 1,663,740 combinations of four building blocks. If an artificial receptor is a spot in a microarray, with 100,000 spots on a slide, the number of slides to contain millions of combinations of building blocks can become unwieldy. Reversible immobilization of building blocks can provide convenient access to several-fold more artificial receptors.

Figure 17 schematically illustrates test ligands with 3, 4, 5, 6, 7, or 8 binding surfaces or environments as polygons with 3, 4, 5, 6, 7, or 8 sides. For small molecules, the number of surfaces or environments may be limited, for example, to 2, 3, or 4. However, for macromolecules the number of surfaces or environments can be significantly larger, for example, 6, 7, or 8. The present invention, through shuffling and exchanging reversibly immobilized building blocks can allow access to large number of combinations of up to, for

example, 8 building blocks in an artificial receptor. Such a process can begin with a convenient number of initial receptors, which can be tested for binding of a test ligand. These artificial receptors can than undergo exchange of additional building blocks until the receptors include up to 8 building blocks. For a set of 81 building blocks, being able to test combinations of 8 building blocks through exchange and shuffling can give practical access to 32 billion artificial receptors (the number of combinations of 8 from a set of 81), without making 32 billion spots in an array.

Embodiments of Artificial Receptors

In an embodiment, the present artificial receptors and methods provide an initial binding event that produces a lead artificial receptor. This lead artificial receptor can then be improved through both shuffling and exchange of receptor substructures. Such compositions and methods employ combinatorial presentation of a large number of receptor building blocks for probing to find a lead artificial receptor. Then, these compositions and methods allow dynamic, spatial redistribution of building blocks for improving binding by the lead artificial receptor.

In an embodiment, reversible mobilization of building blocks on a support provides cooperative interaction of the building blocks with one another and/or with the ligand. This can favor interactive molecular recognition. By way of contrast, conventional dynamic combinatorial libraries (DCL) employ ligand and receptor subunits free in bulk solution. With all components free in bulk solution, each receptor subunit is only held in coordination with the ligand by the weak interactions between the individual subunits and the ligand. In DCL, improvement in binding is limited by dissociation of the building block into the surrounding solution. Thus, the present invention including reversible immobilization of building blocks on a surface provides significant advantages over conventional, solution based DCL.

In an embodiment, cooperative interaction of building blocks and ligand can be envisioned as follows. The ligand can be bound to n building blocks of an artificial receptor. Shuffling can be employed to induce 1 to n-1 of the building blocks to move on the receptor to a different or improved position for binding the ligand or to shuffle away from the ligand. In an embodiment, the ligand can also move and remain bound to one or more building

blocks on the artificial receptor surface. In this manner, the cooperative interaction of building block and ligand can alter or improve ligand binding without the ligand being released from the artificial receptor.

A candidate artificial receptor, a lead artificial receptor, or a working artificial receptor includes combination of building blocks immobilized on, for example, a support. An individual artificial receptor can be a heterogeneous building block spot on a slide or a plurality of building blocks coated on a tube or well.

An array of candidate artificial receptors can be a commercial product sold to parties interested in using the candidate artificial receptors as implements in developing receptors for test ligands of interest. In an embodiment, a useful array of candidate artificial receptors includes a plurality of glass slides, the glass slides including spots of all combinations of members of a set of building blocks, each combination including a predetermined number of building blocks. In an embodiment, a useful group of candidate artificial receptors includes a plurality of tubes or wells, each with a coating of a plurality of immobilized building blocks.

One or more lead artificial receptors can be developed from a plurality of candidate artificial receptors. In an embodiment, a lead artificial receptor includes a combination of building blocks and binds detectable quantities of test ligand upon exposure to, for example, several picomoles of test ligand at a concentration of 1, 0.1, or 0.01 μg/ml, or at 1, 0.1, or 0.01 ng/ml test ligand; at a concentration of 0.01 μg/ml, or at 1, 0.1, or 0.01 ng/ml test ligand; or a concentration of 1, 0.1, or 0.01 ng/ml test ligand.

Artificial receptors, particularly candidate or lead artificial receptors, can be in the form of an array of artificial receptors. Such an array can include, for example, 1.66 million spots, each spot including one combination of 4 building blocks from a set of 81 building blocks. Each spot is a candidate artificial receptor and a combination of building blocks. The array can also be constructed to include lead artificial receptors. For example, the array of artificial receptors can include combinations of fewer building blocks and/or a subset of the building blocks.

In an embodiment, an array of candidate artificial receptors includes building blocks of general Formula 2 (shown hereinbelow), with RE_1 being B1, B2, B3, B3a, B4, B5, B6, B7, B8, or B9 (shown hereinbelow) and with RE_2 being A1, A2, A3, A3a, A4, A5, A6, A7, A8, or A9 (shown hereinbelow). In an embodiment, the framework is tyrosine.

One or more working artificial receptors can be developed from one or more lead artificial receptors. In an embodiment, a working artificial receptor includes a combination of building blocks and binds categorizing or identifying quantities of test ligand upon exposure to, for example, several picomoles of test ligand at a concentration of 100, 10, 1, 0.1, 0.01, or 0.001 ng/ml test ligand; at a concentration of 10, 1, 0.1, 0.01, or 0.001 ng/ml test ligand; or a concentration of 1, 0.1, 0.01, or 0.001 ng/ml test ligand.

In an embodiment, the artificial receptor of the invention includes a plurality of building blocks coupled to a support. In an embodiment, the plurality of building blocks can include or be building blocks of Formula 2 (shown below). In an embodiment, the plurality of building blocks can include or be building blocks of formula TyrA2B2 and/or TyrA4B4 (shown below; the abbreviation for the building block including a linker, a tyrosine framework, and recognition elements AxBy is TyrAxBy). In an embodiment, the plurality of building blocks can include or be building blocks of formula TyrA4B2 and/or TyrA4B4 (shown below). In an embodiment, the plurality of building blocks can include or be building blocks of formula TyrA2B2, TyrA4B2, TyrA4B4, and/or TyrA6B6 (shown below).

In an embodiment, a candidate artificial receptor can include combinations of building blocks of formula TyrA2B2, TyrA4B4, or TyrA6B6. In an embodiment, a candidate artificial receptor can include combinations of building blocks of formula TyrA2B2, TyrA4B4, TyrA6B6, TyrA4B2, or TyrA4B6. In an embodiment, a candidate artificial receptor can include combinations of building blocks of formula TyrA2B2, TyrA2B4, TyrA4B2, TyrA4B4, TyrA4B6, TyrA6B4, TyrA6B6, TyrA6B8, TyrA8B6, or TyrA8B8. In an embodiment, a candidate artificial receptor can include combinations of building blocks of formula TyrA1B1, TyrA2B2, TyrA2B4, TyrA2B6, TyrA2B8, TyrA4B2, TyrA4B4, TyrA4B6, TyrA4B8, TyrA6B2, TyrA6B4, TyrA6B6, TyrA6B8, TyrA8B2, TyrA8B4, TyrA8B6, TyrA8B8, or TyrA9B9.

Working Receptor Systems

In an embodiment, a working artificial receptor or working artificial receptor complex can be incorporated into a system or device for detecting a ligand of interest. Binding of a ligand of interest to a working artificial receptor or complex can produce a detectable signal, for example, through mechanisms and properties such as scattering, absorbing or emitting

light, producing or quenching fluorescence or luminescence, producing or quenching an electrical signal, and the like. Spectroscopic detection methods include use of labels or enzymes to produce light for detection by optical sensors or optical sensor arrays. The light can be ultraviolet, visible, or infrared light, which can be produced and/or detected through fluorescence, fluorescence polarization, chemiluminescence, bioluminescence, or chemibioluminescence. Systems and methods for detecting electrical conduction, and changes in electrical conduction, include ellipsometry, surface plasmon resonance, capacitance, conductometry, surface acoustic wave, quartz crystal microbalance, love-wave, infrared evanescent wave, enzyme labels with electrochemical detection, nanowire field effect transistors, MOSFETS - metal oxide semiconductor field effect transistors, CHEMFETS - organic membrane metal oxide semiconductor field effect transistors, ICP - intrinsically conducting polymers, FRET - fluorescence resonance energy transfer.

Apparatus that can detect such binding to or signal from a working artificial receptor or complex includes UV, visible or infrared spectrometer, fluorescence or luminescence spectrometer, surface plasmon resonance, surface acoustic wave or quartz crystal microbalance detectors, pH, voltammetry or amperometry meters, radioisotope detector, or the like.

In such an apparatus, a working artificial receptor or complex can be positioned on a light fiber to provide a detectable signal, such as an increase or decrease in transmitted light, reflected light, fluorescence, luminescence, or the like. The detectable signal can originate from, for example, a signaling moiety incorporated into the working artificial receptor or complex or a signaling moiety added to the working artificial receptor. The signal can also be intrinsic to the working artificial receptor or to the ligand of interest. The signal can come from, for example, the interaction of the ligand of interest with the working artificial receptor, the interaction of the ligand of interest with a signaling moiety which has been incorporated into the working artificial receptor, into the light fiber, onto the light fiber.

In an embodiment of the system, more than one working artificial receptor, arranged as regions or spots in an array, is on the surface of a support, such as a glass plate. The ligand or ligands of interest or a sample suspected of containing the ligand or ligands of interest (e.g., a sample containing a mixture of DNA segments or fragments, proteins or protein fragments, carbohydrates or carbohydrate fragments, or the like) is brought into

contact with the working artificial receptors or array. Contact can be achieved by addition of a solution of the ligand or ligands of interest or a sample suspected of containing the ligand or ligands of interest. A detectable fluorescence signal can be produced by a signaling moiety incorporated into the working artificial receptor array or a signaling moiety which is added to the ligand or ligands of interest or the sample suspected of containing the ligand or ligands of interest. The fluorescent moieties produce a signal for each working artificial receptor in the array, which produces a pattern of signal response which is characteristic of the composition of the sample of interest.

In an embodiment of the system, more than one working artificial receptor, arranged as regions or spots in an array, is on a support, such as a glass or plastic surface. The surface can be incorporated onto the signaling surfaces of one or more surface plasmon resonance detectors. The ligands of interest or a sample suspected of containing the ligands of interest (e.g., a sample containing a mixture of DNA segments or fragments, proteins or protein fragments, carbohydrates or carbohydrate fragments, or the like) is brought into contact with the working artificial receptors or array. Contacting can be accomplished by addition of a solution of the ligands of interest or a sample suspected of containing the ligands of interest. Detectable electrical signals can be produced by binding of the ligands of interest to the working artificial receptors array on the surface of the surface plasmon resonance detectors. Such detectors produce a signal for each working artificial receptor in the array, which produces a pattern of signal response, which is characteristic of the composition of the sample of interest.

In an embodiment of the system, the working artificial receptor is on a support such as the inner surface of a test tube, microwell, capillary, microchannel, or the like. The ligand of interest or a sample suspected of containing the ligand of interest is brought into contact with the working artificial receptor or complex by addition of a solution containing the ligand of interest or a sample suspected of containing the ligand of interest. A detectable colorimetric, fluorometric, radiometric, or the like, signal is produced by a colorimetric, enzyme, fluorophore, radioisotope, metal ion, or the like, labeled compound or conjugate of the ligand of interest. This labeled moiety can be reacted with the working artificial receptor or complex in competition with the solution containing the ligand of interest or the sample suspected of containing the ligand of interest.

In an embodiment of the system, the working artificial receptor is on a support such as the surface of a surface acoustic wave or quartz crystal microbalance or surface plasmon resonance detector. The ligand of interest or a sample suspected of containing the ligand of interest can be brought into contact with the working artificial receptor or complex by exposure to a stream of air, to an aerosol, or to a solution containing the ligand of interest or a sample suspected of containing the ligand of interest. A detectable electrical signal can be produced by the interaction of the ligand of interest with the working artificial receptor or complex on the active surface of the surface acoustic wave or quartz crystal microbalance or surface plasmon resonance detector.

In an embodiment of the system, the more than one working artificial receptor, arranged as a series of discrete areas or spots or zones or the like, is on the surface of a light fiber. The ligand of interest or a sample suspected of containing the ligand of interest can be brought into contact with the working artificial receptor or complex by exposure to a stream of air, to an aerosol, or to a solution containing the ligand of interest or a sample suspected of containing the ligand of interest. A detectable colorimetric, fluorometric, or like signal can be produced by a label incorporated into the light fiber surface. The colorimetric or fluorogenic signal can be intrinsic to the ligand, or can be an inherent colorimetric or fluorogenic signal produced on binding of the ligand to the working artificial receptors.

An embodiment of the system, combines the artificial receptors with nanotechnology derived nanodevices to give the devices the ability to bind ("see"), bind and incorporate ("eat"), or modify ("use in manufacture") the target material. In an embodiment of the system, the working artificial receptor is incorporated into or on a nanodevice. The ligand of interest or a sample suspected of containing the ligand of interest can be brought into contact with the working artificial receptor nanodevice by addition of the nanodevice to an air or water or soil or biological fluid or cell or biological tissue or biological organism or the like. A detectable signal can be produced by a suitable sensor on the nanodevice and a desired action like a radio signal or chemical reaction or mechanical movement or the like is produced by the nanodevice in response to the ligand of interest.

The present artificial receptors can be part of products used in: analyzing a genome and/or proteome; pharmaceutical development; detectors for any of the test ligands; drug of abuse diagnostics or therapy; hazardous waste analysis or remediation; toxic chemical agent

alert or intervention; disease diagnostics or therapy; cancer diagnostics or therapy; toxic biological agent alert or intervention; food chain contamination analysis or remediation; and the like.

More specifically, the present artificial receptors can be used in products for identification of sequence specific small molecule leads; protein isolation and identification; identification of protein to protein interactions; detecting contaminants in food or food products; clinical analysis of food contaminants; clinical analysis of prostate specific antigen; clinical and field or clinical analysis of cocaine; clinical and field or clinical analysis of other drugs of abuse; other clinical analysis systems, home test systems, or field analysis systems; monitors or alert systems for toxic agents; and the like.

In an embodiment, the present artificial receptors can be employed in studies of proteomics. In such an embodiment, an array of candidate or working artificial receptors can be contacted with a mixture of peptides, polypeptides, and/or proteins. Each mixture can produce a characteristic fingerprint of binding to the array. In addition, identification of a specific receptor environment for a target peptide, polypeptide, and/or protein can be utilized for isolation and analysis of the target. That is, in yet another embodiment, a particular receptor surface can be employed for affinity purification methods, e.g. affinity chromatography.

In an embodiment, the present artificial receptors can be employed to form bioactive surfaces. For example, receptor surfaces can be used to specifically bind antibodies or enzymes.

In an embodiment, the present candidate artificial receptors can be employed to find non-nucleotide artificial receptors for individual DNA or RNA sequences.

In an embodiment, the present candidate artificial receptors can be employed to find receptor surfaces that bind proteins in a certain configuration or orientation. Many proteins (e.g. antibodies, enzymes, receptors) are stable and/or active in specific environments. Defined receptor surfaces can be used to produce binding environments that selectively retain or orient the protein for maximum stability and/or activity.

In an embodiment, the present candidate artificial receptors can be employed to find artificial receptors that do not bind selected molecules or compositions or that exhibit low friction. For example, an array of candidate artificial receptors can be surveyed to find

artificial receptors that not bind to complex biological mixtures like blood serum. Non-binding surfaces can be made by coating with the selected artificial receptor. For example, surfaces can be made that are anti-filming or that have antimicrobial properties.

In an embodiment, the present candidate artificial receptors can be employed to find receptor surfaces that provide a spatially oriented binding surface for a stereospecific reaction. For example, an artificial receptor surface can bind a small molecule with particular functional groups exposed to the environment, and others obscured by the receptor. Such an artificial receptor surface can be employed in synthesis including chiral induction. For example, a substrate (e.g. a steroid) can be stereospecifically bound to the artificial receptor and present a particular moiety/sub-structure/"face" for reaction with a reagent in solution. Similarly, the artificial receptor surface can act as a protecting group where a reactive moiety of a molecule is "protected" by binding to the receptor surface so that a different moiety with similar reactivity can be transformed.

In an embodiment, the present candidate artificial receptors can be employed to find artificial receptors or receptor surfaces that act as an artificial enzyme. For example, such a receptor surface can be utilized as co-factor to bind a catalytic center and/or to orient the substrate for reaction.

In an embodiment, the present artificial receptors can be employed to form selective membranes. Such a selective membrane can be based on a molecular gate including an artificial receptor surface. For example, an artificial receptor surface can line the walls of pores in the membrane and either allow or block a target molecule from passing through the pores. For example, an artificial receptor surface can line the walls of pores in the membrane and act as "gatekeepers" on e.g. microcantilevers/molecular cantilevers to allow gate opening or closing on binding of the target.

In an embodiment, the present candidate artificial receptors can be employed to find artificial receptors for use on surfaces as intelligent materials. For example, the artificial receptor surface can act as a molecular electronic switch. In such a switch, binding of a target, which can be either an organic or an inorganic moiety, can act as an on/off gate for electron or ion flow.

Test Ligands

The test ligand can be any ligand for which binding to an array or surface can be detected. The test ligand can be a pure compound, a mixture, or a "dirty" mixture containing a natural product or pollutant. Such dirty mixtures can be tissue homogenate, biological fluid, soil sample, water sample, or the like.

Test ligands include prostate specific antigen, other cancer markers, insulin, warfarin, other anti-coagulants, cocaine, other drugs-of-abuse, markers for *E. coli*, markers for *Salmonella* sp., markers for other food-borne toxins, food-borne toxins, markers for Smallpox virus, markers for anthrax, markers for other possible toxic biological agents, pharmaceuticals and medicines, pollutants and chemicals in hazardous waste, toxic chemical agents, markers of disease, pharmaceuticals, pollutants, biologically important cations (e.g., potassium or calcium ion), peptides, carbohydrates, enzymes, bacteria, viruses, mixtures thereof, and the like. In certain embodiments, the test ligand can be at least one of small organic molecules, inorganic/organic complexes, metal ion, mixture of proteins, protein, nucleic acid, mixture of nucleic acids, mixtures thereof, and the like.

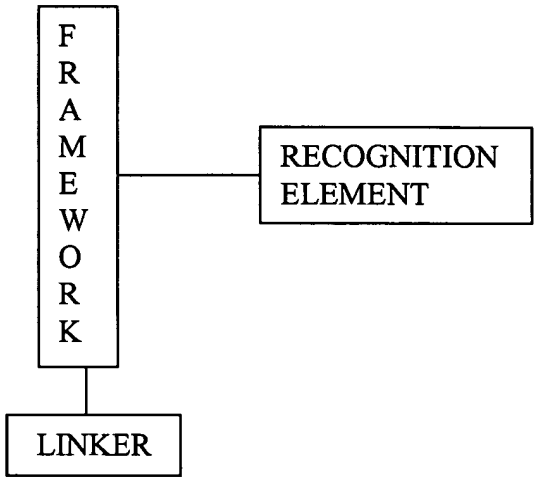
Building Blocks

The present invention relates to building blocks for making or forming candidate artificial receptors. Building blocks are designed, made, and selected to provide a variety of structural characteristics among a small number of compounds. A building block can provide one or more structural characteristics such as positive charge, negative charge, acid, base, electron acceptor, electron donor, hydrogen bond donor, hydrogen bond acceptor, free electron pair, π electrons, charge polarization, hydrophilicity, hydrophobicity, and the like. A building block can be bulky or it can be small.

A building block can be visualized as including several components, such as one or more frameworks, one or more linkers, and/or one or more recognition elements. The framework can be covalently coupled to each of the other building block components. The linker can be covalently coupled to the framework and to a support. The recognition element can be covalently coupled to the framework. In an embodiment, a building block includes a framework, a linker, and a recognition element. In an embodiment, a building block includes a framework, a linker, and two recognition elements.

The present building blocks can also include a functional group or structural feature or moiety that allows them to be reversibly immobilized on a support, e.g., by way of a lawn. For example, the linker can be covalently coupled to the framework and reversibly coupled to a support or to a lawn molecule.

A building block including a framework, a linker, and one or more recognition elements can be schematically represented as:

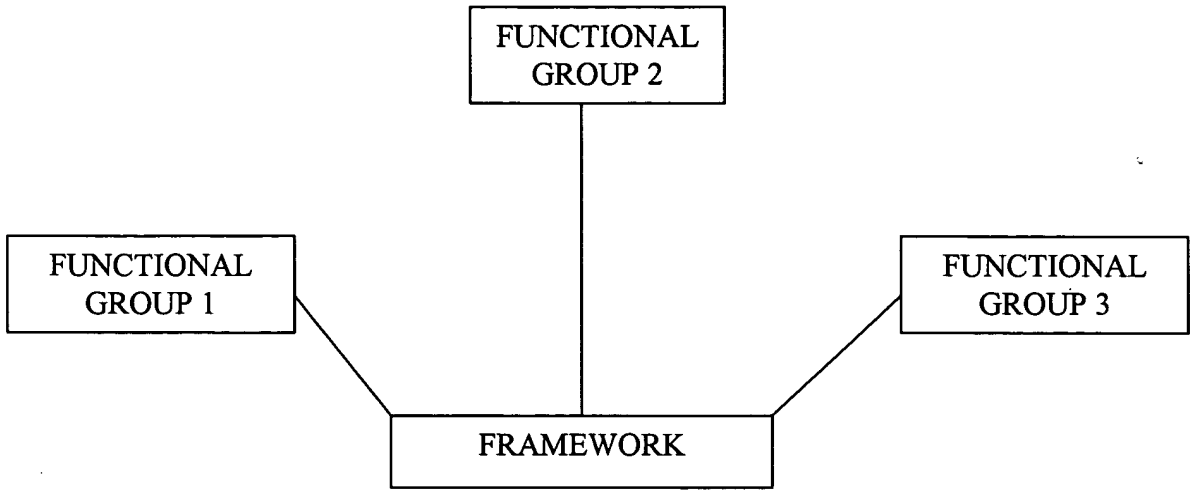


Scheme 1

Framework

The framework can be selected for functional groups that provide for coupling to the recognition moiety and for coupling to or being the linking moiety. The framework can interact with the ligand as part of the artificial receptor. For example, the framework can include multiple reaction sites with orthogonal and reliable functional groups and with controlled stereochemistry. Suitable functional groups with orthogonal and reliable chemistries include, for example, carboxyl, amine, hydroxyl, phenol, carbonyl, and thiol groups, which can be individually protected, deprotected, and derivatized. For example, the framework can have two, three, or four functional groups with orthogonal and reliable chemistries.

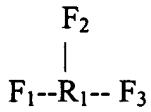
A framework including three sites for orthogonal and reliable chemistries can be schematically represented as:



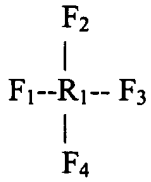
Scheme 2

The three functional groups can be independently selected, for example, from carboxyl, amine, hydroxyl, phenol, carbonyl, or thiol group. The framework can include alkyl, substituted alkyl, cycloalkyl, heterocyclic, substituted heterocyclic, aryl alkyl, aryl, heteroaryl, heteroaryl alkyl, and like moieties.

A general structure for a framework with three functional groups can be represented by Formula 1a:



A general structure for a framework with four functional groups can be represented by Formula 1b:



In these general structures: R_1 can be a 1-12, 1-6, or 1-4 carbon alkyl, substituted alkyl, cycloalkyl, heterocyclic, substituted heterocyclic, aryl alkyl, aryl, heteroaryl, heteroaryl alkyl, or like group; and F_1, F_2, F_3, or F_4 can independently be a carboxyl, amine, hydroxyl, phenol, carbonyl, or thiol group. F_1, F_2, F_3, or F_4 can independently be a 1-12, 1-6, or 1-4 carbon alkyl, substituted alkyl, cycloalkyl, heterocyclic, substituted heterocyclic, aryl alkyl, aryl, heteroaryl, heteroaryl alkyl, or inorganic group substituted with carboxyl, amine, hydroxyl, phenol, carbonyl, or thiol group. F_3 and/or F_4 can be absent.

A variety of compounds fit the schemes and formulas describing the framework including amino acids, and naturally occurring or synthetic compounds including, for example, oxygen and sulfur functional groups. The compounds can be racemic, optically active, or achiral. For example, the compounds can be natural or synthetic amino acids, α-hydroxy acids, thioic acids, and the like.

Suitable molecules for use as a framework include a natural or synthetic amino acid, particularly an amino acid with a functional group (e.g., third functional group) on its side chain. Amino acids include carboxyl and amine functional groups. The side chain functional group can include, for natural amino acids, an amine (e.g., alkyl amine, heteroaryl amine), hydroxyl, phenol, carboxyl, thiol, thioether, or amidino group. Natural amino acids suitable for use as frameworks include, for example, serine, threonine, tyrosine, aspartic acid, glutamic acid, asparagine, glutamine, cysteine, lysine, arginine, histidine. Synthetic amino acids can include the naturally occurring side chain functional groups or synthetic side chain functional groups which modify or extend the natural amino acids with alkyl, substituted alkyl, cycloalkyl, heterocyclic, substituted heterocyclic, aryl alkyl, aryl, heteroaryl, heteroaryl alkyl, and like moieties as framework and with carboxyl, amine, hydroxyl, phenol, carbonyl, or thiol functional groups. Suitable synthetic amino acids include β-amino acids and homo or β analogs of natural amino acids.

Suitable framework amino acids include serine, threonine, or tyrosine, e.g., serine or tyrosine, e.g., tyrosine. Figure 18 illustrates serine as a framework for a building block and reactions for forming building blocks from serine, tyrosine, and other amino acids. Threonine and tyrosine can exhibit reactivity similar to serine. Advantageously, serine, threonine, and tyrosine include: 1) multiple, orthogonal, well characterized reaction sites, 2) known methods and reactions for application as a combinatorial framework, 3) diversity of

sub-structures and domains which can be incorporated through the carboxyl, α-amine, and hydroxyl functionalities, 4) compact distribution of the multiple reaction sites around a tetrahedral carbon framework, and 5) ready commercial availability of reagents for forming linkers and/or recognition elements.

Figure 19 illustrates configurations in which recognition element, linker, and a chiral element can be coupled to a tyrosine framework. Threonine and serine can form analogous configurations. The chiral element is a substituent that renders the carbon atom to which it is attached a chiral center. When one or more different recognition elements are also substituents on or coupled to the chiral center, the recognition elements can adopt two or more enantiomeric configurations. Such enantiomers can be advantageous for providing diversity among building blocks.

Although not limiting to the present invention, a framework amino acid, such as serine, threonine, or tyrosine, with a linker and two recognition elements can be visualized with one of the recognition elements in a pendant orientation and the other in an equatorial orientation, relative to the extended carbon chain of the framework.

Although not limiting to the present invention, the present building block framework can include: 1) diversity of framework reaction sites to maximize incorporation of potential receptor functionality, 2) reliable reaction and protection chemistries, 3) compact structure, 4) incorporation of diverse sub-structures (e.g., recognition elements), 5) a suitable platform for linker element incorporation, and/or 6) development of non-equivalent diversity domains to minimize redundancy in the receptor building blocks while maximizing the number of functional groups and sub-structures incorporated into a small library. In an embodiment, the framework includes multiple reaction sites with compact format. Compact format is advantageous for providing a building block that fits at a suitable density on a support.

All of the naturally occurring and many synthetic amino acids are commercially available. Further, forms of these amino acids derivatized or protected to be suitable for reactions for coupling to recognition element(s) and/or linkers can be purchased or made by known methods (see, e.g., Green, TW; Wuts, PGM (1999), Protective Groups in Organic Synthesis Third Edition, Wiley-Interscience, New York, 779 pp.; Bodanszky, M.; Bodanszky, A. (1994), The Practice of Peptide Synthesis Second Edition, Springer-Verlag, New York, 217 pp.).

Suitable reaction schemes for preparing amino acids for reactions for forming building blocks according to the present invention include those provided in the present Examples.

Recognition Element

The recognition element can be selected to provide one or more structural characteristics to the building block. The framework can interact with the ligand as part of the artificial receptor. For example, the recognition element can provide one or more structural characteristics such as positive charge, negative charge, acid, base, electron acceptor, electron donor, hydrogen bond donor, hydrogen bond acceptor, free electron pair, π electrons, charge polarization, hydrophilicity, hydrophobicity, and the like. A recognition element can be a small group or it can be bulky.

In an embodiment the recognition element can be a 1-12, 1-6, or 1-4 carbon alkyl, substituted alkyl, cycloalkyl, heterocyclic, substituted heterocyclic, aryl alkyl, aryl, heteroaryl, heteroaryl alkyl, or like group. The recognition element can be substituted with a group that includes or imparts positive charge, negative charge, acid, base, electron acceptor, electron donor, hydrogen bond donor, hydrogen bond acceptor, free electron pair, π electrons, charge polarization, hydrophilicity, hydrophobicity, and the like.

Recognition elements with a positive charge (e.g., at neutral pH in aqueous compositions) include amines, quaternary ammonium moieties, sulfonium, phosphonium, ferrocene, and the like. Suitable amines include alkyl amines, alkyl diamines, heteroalkyl amines, aryl amines, heteroaryl amines, aryl alkyl amines, pyridines, heterocyclic amines (saturated or unsaturated, the nitrogen in the ring or not), amidines, hydrazines, and the like. Alkyl amines generally have 1 to 12 carbons, e.g., 1-8 carbons, rings can have 3-12 carbons, e.g., 3-8 carbons. Suitable alkyl amines include that of formula B9. Suitable heterocyclic or alkyl heterocyclic amines include that of formula A9. Suitable pyridines include those of formulas A5 and B5. Any of the amines can be employed as a quaternary ammonium compound. Additional suitable quaternary ammonium moieties include trimethyl alkyl quaternary ammonium moieties, dimethyl ethyl alkyl quaternary ammonium moieties, dimethyl alkyl quaternary ammonium moieties, aryl alkyl quaternary ammonium moieties, pyridinium quaternary ammonium moieties, and the like.

Recognition elements with a negative charge (e.g., at neutral pH in aqueous compositions) include carboxylates, phenols substituted with strongly electron withdrawing groups (e.g., substituted tetrachlorophenols), phosphates, phosphonates, phosphinates, sulphates, sulphonates, thiocarboxylates, and hydroxamic acids. Suitable carboxylates include alkyl carboxylates, aryl carboxylates, and aryl alkyl carboxylates. Suitable phosphates include phosphate mono-, di-, and tri- esters, and phosphate mono-, di-, and tri-amides. Suitable phosphonates include phosphonate mono- and di- esters, and phosphonate mono- and di- amides (e.g., phosphonamides). Suitable phosphinates include phosphinate esters and amides.

Recognition elements with a negative charge and a positive charge (at neutral pH in aqueous compositions) include sulfoxides, betaines, and amine oxides.

Acidic recognition elements can include carboxylates, phosphates, sulphates, and phenols. Suitable acidic carboxylates include thiocarboxylates. Suitable acidic phosphates include the phosphates listed hereinabove.

Basic recognition elements include amines. Suitable basic amines include alkyl amines, aryl amines, aryl alkyl amines, pyridines, heterocyclic amines (saturated or unsaturated, the nitrogen in the ring or not), amidines, and any additional amines listed hereinabove. Suitable alkyl amines include that of formula B9. Suitable heterocyclic or alkyl heterocyclic amines include that of formula A9. Suitable pyridines include those of formulas A5 and B5.

Recognition elements including a hydrogen bond donor include amines, amides, carboxyls, protonated phosphates, protonated phosphonates, protonated phosphinates, protonated sulphates, protonated sulphinates, alcohols, and thiols. Suitable amines include alkyl amines, aryl amines, aryl alkyl amines, pyridines, heterocyclic amines (saturated or unsaturated, the nitrogen in the ring or not), amidines, ureas, and any other amines listed hereinabove. Suitable alkyl amines include that of formula B9. Suitable heterocyclic or alkyl heterocyclic amines include that of formula A9. Suitable pyridines include those of formulas A5 and B5. Suitable protonated carboxylates, protonated phosphates include those listed hereinabove. Suitable amides include those of formulas A8 and B8. Suitable alcohols include primary alcohols, secondary alcohols, tertiary alcohols, and aromatic alcohols (e.g.,

phenols). Suitable alcohols include those of formulas A7 (a primary alcohol) and B7 (a secondary alcohol).

Recognition elements including a hydrogen bond acceptor or one or more free electron pairs include amines, amides, carboxylates, carboxyl groups, phosphates, phosphonates, phosphinates, sulphates, sulphonates, alcohols, ethers, thiols, and thioethers. Suitable amines include alkyl amines, aryl amines, aryl alkyl amines, pyridines, heterocyclic amines (saturated or unsaturated, the nitrogen in the ring or not), amidines, ureas, and amines as listed hereinabove. Suitable alkyl amines include that of formula B9. Suitable heterocyclic or alkyl heterocyclic amines include that of formula A9. Suitable pyridines include those of formulas A5 and B5. Suitable carboxylates include those listed hereinabove. Suitable amides include those of formulas A8 and B8. Suitable phosphates, phosphonates and phosphinates include those listed hereinabove. Suitable alcohols include primary alcohols, secondary alcohols, tertiary alcohols, aromatic alcohols, and those listed hereinabove. Suitable alcohols include those of formulas A7 (a primary alcohol) and B7 (a secondary alcohol). Suitable ethers include alkyl ethers, aryl alkyl ethers. Suitable alkyl ethers include that of formula A6. Suitable aryl alkyl ethers include that of formula A4. Suitable thioethers include that of formula B6.

Recognition elements including uncharged polar or hydrophilic groups include amides, alcohols, ethers, thiols, thioethers, esters, thio esters, boranes, borates, and metal complexes. Suitable amides include those of formulas A8 and B8. Suitable alcohols include primary alcohols, secondary alcohols, tertiary alcohols, aromatic alcohols, and those listed hereinabove. Suitable alcohols include those of formulas A7 (a primary alcohol) and B7 (a secondary alcohol). Suitable ethers include those listed hereinabove. Suitable ethers include that of formula A6. Suitable aryl alkyl ethers include that of formula A4.

Recognition elements including uncharged hydrophobic groups include alkyl (substituted and unsubstituted), alkene (conjugated and unconjugated), alkyne (conjugated and unconjugated), aromatic. Suitable alkyl groups include lower alkyl, substituted alkyl, cycloalkyl, aryl alkyl, and heteroaryl alkyl. Suitable lower alkyl groups include those of formulas A1, A3, A3a, and B1. Suitable aryl alkyl groups include those of formulas A3, A3a, A4, B3, B3a, and B4. Suitable alkyl cycloalkyl groups include that of formula B2. Suitable alkene groups include lower alkene and aryl alkene. Suitable aryl alkene groups

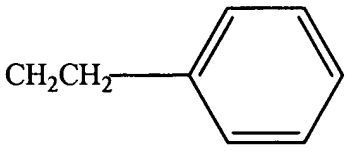
include that of formula B4. Suitable aromatic groups include unsubstituted aryl, heteroaryl, substituted aryl, aryl alkyl, heteroaryl alkyl, alkyl substituted aryl, and polyaromatic hydrocarbons. Suitable aryl alkyl groups include those of formulas A3, A3a and B4. Suitable alkyl heteroaryl groups include those of formulas A5 and B5.

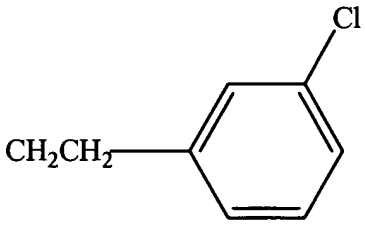
Spacer (e.g., small) recognition elements include hydrogen, methyl, ethyl, and the like. Bulky recognition elements include 7 or more carbon or hetero atoms.

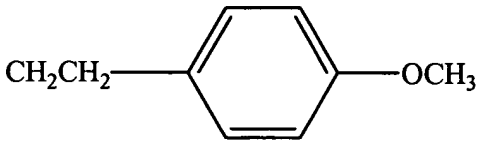
Formulas A1-A9 and B1-B9 are:

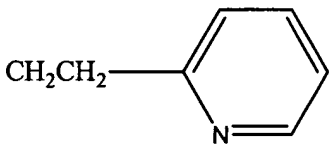
$CH_2\ CH_3$ A1

$CH_2CH(CH_3)_2$ A2

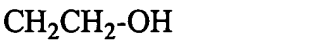
 A3

 A3a

 A4

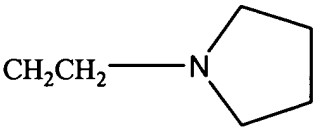
 A5

CH_2CH_2-O-CH_3 A6



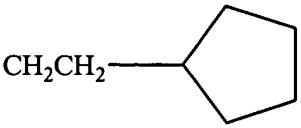
A7

A8

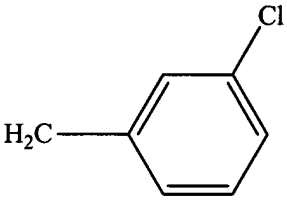


A9

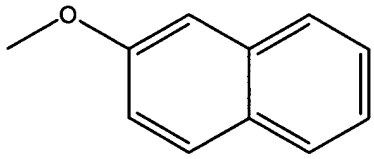
B1



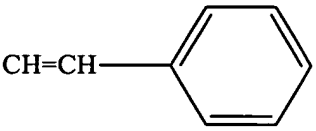
B2



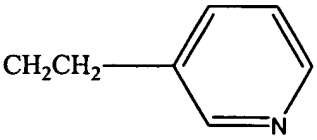
B3



B3a



B4



B5

CH_2-S-CH_3	B6
$CH_2CH(OH)CH_3$	B7
$CH_2CH_2C(O)$-NH_2	B8
$CH_2CH_2CH_2$-N-$(CH_3)_2$	B9

These A and B recognition elements can be called derivatives of, according to a standard reference: A1, ethylamine; A2, isobutylamine; A3, phenethylamine; A4, 4-methoxyphenethylamine; A5, 2-(2-aminoethyl)pyridine; A6, 2-methoxyethylamine; A7, ethanolamine; A8, N-acetylethylenediamine; A9, 1-(2-aminoethyl)pyrrolidine; B1, acetic acid, B2, cyclopentylpropionic acid; B3, 3-chlorophenylacetic acid; B4, cinnamic acid; B5, 3-pyridinepropionic acid; B6, (methylthio)acetic acid; B7, 3-hydroxybutyric acid; B8, succinamic acid; and B9, 4-(dimethylamino)butyric acid.

In an embodiment, the recognition elements include one or more of the structures represented by formulas A1, A2, A3, A3a, A4, A5, A6, A7, A8, and/or A9 (the A recognition elements) and/or B1, B2, B3, B3a, B4, B5, B6, B7, B8, and/or B9 (the B recognition elements). In an embodiment, each building block includes an A recognition element and a B recognition element. In an embodiment, a group of 81 such building blocks includes each of the 81 unique combinations of an A recognition element and a B recognition element. In an embodiment, the A recognition elements are linked to a framework at a pendant position. In an embodiment, the B recognition elements are linked to a framework at an equatorial position. In an embodiment, the A recognition elements are linked to a framework at a pendant position and the B recognition elements are linked to the framework at an equatorial position.

Although not limiting to the present invention, it is believed that the A and B recognition elements represent the assortment of functional groups and geometric configurations employed by polypeptide receptors. Although not limiting to the present invention, it is believed that the A recognition elements represent six advantageous functional groups or configurations and that the addition of functional groups to several of the aryl groups increases the range of possible binding interactions. Although not limiting to

the present invention, it is believed that the B recognition elements represent six advantageous functional groups, but in different configurations than employed for the A recognition elements. Although not limiting to the present invention, it is further believed that this increases the range of binding interactions and further extends the range of functional groups and configurations that is explored by molecular configurations of the building blocks.

The above characterization of molecular configurations is not intended to be limiting to the present invention. Each of the illustrated molecular configurations can bind any of a variety of test ligands, but as illustrated can also be envisioned for particular configurations of test ligand.

In an embodiment, the building blocks including the A and B recognition elements can be visualized as occupying a binding space defined by lipophilicity/hydrophilicity and volume. A volume can be calculated (using known methods) for each building block including the various A and B recognition elements. A measure of lipophilicity/hydrophilicity (logP) can be calculated (using known methods) for each building block including the various A and B recognition elements. Negative values of logP show affinity for water over nonpolar organic solvent and indicate a hydrophilic nature. A plot of volume versus logP can then show the distribution of the building blocks through a binding space defined by size and lipophilicity/hydrophilicity.

Figure 7 schematically illustrates binding space divided qualitatively into 4 quadrants - large hydrophilic, large hydrophobic, small hydrophilic, and small lipophilic. Figure 7 denotes a small triangle of the large hydrophilic quadrant as very large and highly hydrophilic. Figure 7 denotes a small triangle of the small lipophilic quadrant as very small and highly lipophilic.

Figure 8 illustrates a plot of volume versus logP for 81 building blocks including each of the 9 A and 9 B recognition elements. This plot illustrates that the 81 building blocks with A and B recognition elements fill a significant portion of the binding space defined by volume and lipophilicity/hydrophilicity. The space filled by the 81 building blocks is roughly bounded by the A1B1, A2B2, ... A9B9 building blocks (Figure 8). The 81 building blocks with A and B recognition elements fill a majority of this binding space excluding only

the portion denoted very large and highly hydrophilic and the portion denoted very small and highly lipophilic.

Figures 9A and 9B illustrate a plot of volume versus logP for combinations of building blocks with A and B recognition elements forming candidate artificial receptors. The volumes and values of logP for these candidate artificial receptors generally fill in the space occupied by the individual building blocks. Figure 9B represents a detail from Figure 9A. This detail illustrates that the candidate artificial receptors fill the binding space evenly. Candidate artificial receptors made from building blocks with A and B recognition elements include receptors with a wide range of sizes and a wide range of values of lipophilicity/hydrophilicity.

Figure 10 illustrates that candidate artificial receptors made up of building blocks can be sorted and evaluated with respect to their nearest neighbors, other candidate artificial receptors made up of one or more of the same building blocks. In an embodiment, the nearest neighbor can be made up of a subset of the building blocks forming the subject candidate artificial receptor. For example, as shown in Figure 10, a candidate artificial receptor made up of TyrA3B3/TyrA4B4/TyrA5B5/TyrA6B6 has among its nearest neighbors candidate artificial receptors TyrA4B4/TyrA5B5/TyrA6B6, TyrA3B3/TyrA5B5/TyrA6B6, TyrA3B3/TyrA4B4/TyrA6B6, and TyrA3B3/TyrA4B4/TyrA5B5. These candidate artificial receptors in turn have additional nearest neighbors. Candidate receptors and/or recognition elements can also be grouped as neighbors based on lipophilicity/hydrophilicity, size, charge, or another physical or chemical characteristic.

Reagents that form many of the recognition elements are commercially available. For example, reagents for forming recognition elements A1, A2, A3, A3a, A4, A5, A6, A7, A8, A9 B1, B2, B3, B3a, B4, B5, B6, B7, B8, and B9 are commercially available.

Linkers

Linkers for Reversibly Immobilizable Building Blocks

The linker is selected to provide suitable reversible immobilization of the building block on a support or lawn. The linker can interact with the ligand as part of the artificial receptor. The linker can also provide bulk, distance from the support, hydrophobicity, hydrophilicity, and like structural characteristics to the building block. In an embodiment,

the linker forms a covalent bond with a functional group on the framework. In an embodiment, the linker also includes a functional group that can reversibly interact with the support or lawn, e.g., through reversible covalent bonding or noncovalent interactions.

In an embodiment, the linker includes one or more moieties that can engage in reversible covalent bonding. Suitable groups for reversible covalent bonding include those described hereinabove. An artificial receptor can include building blocks reversibly immobilized on the lawn or support through, for example, imine, acetal, ketal, disulfide, ester, or like linkages. Such functional groups can engage in reversible covalent bonding. Such a functional group can be referred to as a covalent bonding moiety, e.g., a second covalent bonding moiety.

In an embodiment, the linker can be functionalized with moieties that can engage in noncovalent interactions. For example, the linker can include functional groups such as an ionic group, a group that can hydrogen bond, or a group that can engage in van der Waals or other hydrophobic interactions. Such functional groups can include cationic groups, anionic groups, lipophilic groups, amphiphilic groups, and the like.

In an embodiment, the present methods and compositions can employ a linker including a charged moiety (e.g., a second charged moiety). Suitable charged moieties include positively charged moieties and negatively charged moieties. Suitable positively charged moieties include amines, quaternary ammonium moieties, sulfonium, phosphonium, ferrocene, and the like. Suitable negatively charged moieties (e.g., at neutral pH in aqueous compositions) include carboxylates, phenols substituted with strongly electron withdrawing groups (e.g., tetrachlorophenols), phosphates, phosphonates, phosphinates, sulphates, sulphonates, thiocarboxylates, and hydroxamic acids.

In an embodiment, the present methods and compositions can employ a linker including a group that can hydrogen bond, either as donor or acceptor (e.g., a second hydrogen bonding group). For example, the linker can include one or more carboxyl groups, amine groups, hydroxyl groups, carbonyl groups, or the like. Ionic groups can also participate in hydrogen bonding.

In an embodiment, the present methods and compositions can employ a linker including a lipophilic moiety (e.g., a second lipophilic moiety). Suitable lipophilic moieties include one or more branched or straight chain C_{6-36} alkyl, C_{8-24} alkyl, C_{12-24} alkyl, C_{12-18}

alkyl, or the like; C_{6-36} alkenyl, C_{8-24} alkenyl, C_{12-24} alkenyl, C_{12-18} alkenyl, or the like, with, for example, 1 to 4 double bonds; C_{6-36} alkynyl, C_{8-24} alkynyl, C_{12-24} alkynyl, C_{12-18} alkynyl, or the like, with, for example, 1 to 4 triple bonds; chains with 1-4 double or triple bonds; chains including aryl or substituted aryl moieties (e.g., phenyl or naphthyl moieties at the end or middle of a chain); polyaromatic hydrocarbon moieties; cycloalkane or substituted alkane moieties with numbers of carbons as described for chains; combinations or mixtures thereof; or the like. The alkyl, alkenyl, or alkynyl group can include branching; within chain functionality like an ether group; terminal functionality like alcohol, amide, carboxylate or the like; or the like. In an embodiment the linker includes or is a lipid, such as a phospholipid. In an embodiment, the lipophilic moiety includes or is a 12-carbon aliphatic moiety.

In an embodiment, the linker includes a lipophilic moiety (e.g., a second lipophilic moiety) and a covalent bonding moiety (e.g., a second covalent bonding moiety). In an embodiment, the linker includes a lipophilic moiety (e.g., a second lipophilic moiety) and a charged moiety (e.g., a second charged moiety).

In an embodiment, the linker forms or can be visualized as forming a covalent bond with an alcohol, phenol, thiol, amine, carbonyl, or like group on the framework. Between the bond to the framework and the group participating in or formed by the reversible interaction with the support or lawn, the linker can include an alkyl, substituted alkyl, cycloalkyl, heterocyclic, substituted heterocyclic, aryl alkyl, aryl, heteroaryl, heteroaryl alkyl, ethoxy or propoxy oligomer, a glycoside, or like moiety.

For example, suitable linkers can include: the functional group participating in or formed by the bond to the framework, the functional group or groups participating in or formed by the reversible interaction with the support or lawn, and a linker backbone moiety. The linker backbone moiety can include about 4 to about 48 carbon or heteroatoms, about 8 to about 14 carbon or heteroatoms, about 12 to about 24 carbon or heteroatoms, about 16 to about 18 carbon or heteroatoms, about 4 to about 12 carbon or heteroatoms, about 4 to about 8 carbon or heteroatoms, or the like. The linker backbone can include an alkyl, substituted alkyl, cycloalkyl, heterocyclic, substituted heterocyclic, aryl alkyl, aryl, heteroaryl, heteroaryl alkyl, ethoxy or propoxy oligomer, a glycoside, mixtures thereof, or like moiety.

In an embodiment, the linker includes a lipophilic moiety, the functional group participating in or formed by the bond to the framework, and, optionally, one or more moieties for forming a reversible covalent bond, a hydrogen bond, or an ionic interaction. In such an embodiment, the lipophilic moiety can have about 4 to about 48 carbons, about 8 to about 14 carbons, about 12 to about 24 carbons, about 16 to about 18 carbons, or the like. In such an embodiment, the linker can include about 1 to about 8 reversible bond/interaction moieties or about 2 to about 4 reversible bond/interaction moieties. Suitable linkers have structures such as $(CH_2)_nCOOH$, with n=12-24, n=17-24, or n=16-18.

Additional Embodiments of Linkers

The linker is selected to provide a suitable covalent attachment of the building block to a support. The framework can interact with the ligand as part of the artificial receptor. The linker can also provide bulk, distance from the support, hydrophobicity, hydrophilicity, and like structural characteristics to the building block. In an embodiment, linker forms a covalent bond with a functional group on the framework. In an embodiment, before attachment to the support the linker also includes a functional group that can be activated to react with or that will react with a functional group on the support. In an embodiment, once attached to the support, the linker forms a covalent bond with the support and with the framework.

In an embodiment, the linker forms or can be visualized as forming a covalent bond with an alcohol, phenol, thiol, amine, carbonyl, or like group on the framework. The linker can include a carboxyl, alcohol, phenol, thiol, amine, carbonyl, maleimide, or like group that can react with or be activated to react with the support. Between the bond to the framework and the group formed by the attachment to the support, the linker can include an alkyl, substituted alkyl, cycloalkyl, heterocyclic, substituted heterocyclic, aryl alkyl, aryl, heteroaryl, heteroaryl alkyl, ethoxy or propoxy oligomer, a glycoside, or like moiety.

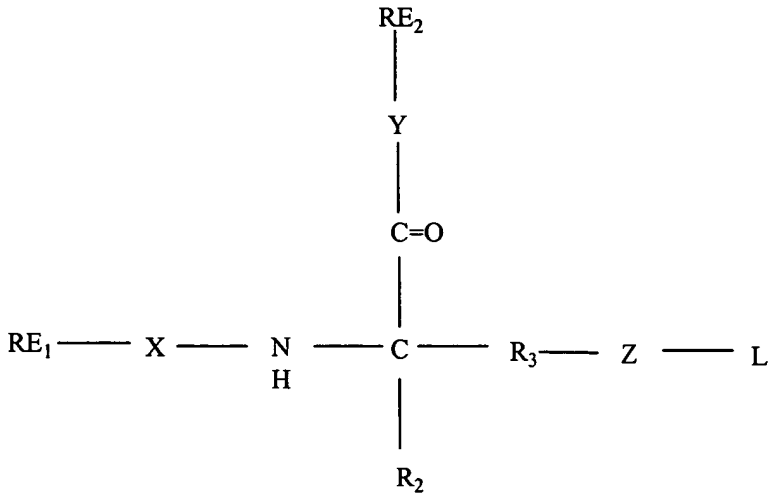
The linker can include a good leaving group bonded to, for example, an alkyl or aryl group. The leaving group being "good" enough to be displaced by the alcohol, phenol, thiol, amine, carbonyl, or like group on the framework. Such a linker can include a moiety represented by the formula: R-X, in which X is a leaving group such as halogen (e.g., -Cl, -Br or -I), tosylate, mesylate, triflate, and R is alkyl, substituted alkyl, cycloalkyl,

heterocyclic, substituted heterocyclic, aryl alkyl, aryl, heteroaryl, heteroaryl alkyl, ethoxy or propoxy oligomer, a glycoside, or like moiety.

Suitable linker groups include those of formula: $(CH_2)_nCOOH$, with n=1-16, n=2-8, n=2-6, or n=3. Reagents that form suitable linkers are commercially available and include any of a variety of reagents with orthogonal functionality.

Embodiments of Building Blocks

In an embodiment, building blocks can be represented by Formula 2:



in which: RE_1 is recognition element 1, RE_2 is recognition element 2, and L is a linker. X is absent, C=O, CH_2, NR, NR_2, NH, NHCONH, SCONH, CH=N, or OCH_2NH. In certain embodiments, X is absent or C=O. Y is absent, NH, O, CH_2, or NRCO. In certain embodiments, Y is NH or O. In an embodiment, Y is NH. Z can be CH2, O, NH, S, CO, NR, NR_2, NHCONH, SCONH, CH=N, or OCH_2NH. In an embodiment, Z is O. R_2 can be H, CH_3, or another group that confers chirality on the building block and has size similar to or smaller than a methyl group. R_3 is CH_2; CH_2-phenyl; $CHCH_3$; $(CH_2)_n$ with n=2-3; or cyclic alkyl with 3-8 carbons, e.g., 5-6 carbons, phenyl, naphthyl. In certain embodiments, R_3 is CH_2 or CH_2-phenyl.

RE_1 is B1, B2, B3, B3a, B4, B5, B6, B7, B8, B9, A1, A2, A3, A3a, A4, A5, A6, A7, A8, or A9. In an embodiment, RE_1 is B1, B2, B3, B3a, B4, B5, B6, B7, B8, or B9. RE_2 is A1, A2, A3, A3a, A4, A5, A6, A7, A8, A9, B1, B2, B3, B3a, B4, B5, B6, B7, B8, or B9. In an embodiment, RE_2 is A1, A2, A3, A3a, A4, A5, A6, A7, A8, or A9. In an embodiment, RE_1 can be B2, B4, or B6 and RE_2 can be A2, A4, or A6. In an embodiment, RE_1 can be B1,

B3, B3a, B6, or B8 and RE_2 can be A2, A4, A5, or A9. In an embodiment, RE_1 can be B2, B4, B6, or B8 and RE_2 can be A2, A4, A6, or A8. In an embodiment, RE_1 can be B1, B2, B4, B6, or B8 and RE_2 can be A1, A2, A4, A6, or A8. In an embodiment, RE_1 can be B1, B2, B4, B6, B8, or B9 and RE_2 can be A1, A2, A4, A6, A8, or A9. In an embodiment, RE_1 can be B2, B3a, B4, B5, B6, B7, or B8. In an embodiment, RE_2 can be A2, A3a, A4, A5, A6, A7, or A8.

In an embodiment, L is the functional group participating in or formed by the bond to the framework (such groups are described herein), the functional group or groups participating in or formed by the reversible interaction with the support or lawn (such groups are described herein), and a linker backbone moiety. In an embodiment, the linker backbone moiety is about 4 to about 48 carbon or heteroatom alkyl, substituted alkyl, cycloalkyl, heterocyclic, substituted heterocyclic, aryl alkyl, aryl, heteroaryl, heteroaryl alkyl, ethoxy or propoxy oligomer, a glycoside, or mixtures thereof; or about 8 to about 14 carbon or heteroatoms, about 12 to about 24 carbon or heteroatoms, about 16 to about 18 carbon or heteroatoms, about 4 to about 12 carbon or heteroatoms, about 4 to about 8 carbon or heteroatoms.

In an embodiment, the L is the functional group participating in or formed by the bond to the framework (such groups are described herein) and a lipophilic moiety (such groups are described herein) of about 4 to about 48 carbons, about 8 to about 14 carbons, about 12 to about 24 carbons, about 16 to about 18 carbons. In an embodiment, this L also includes about 1 to about 8 reversible bond/interaction moieties (such groups are described herein) or about 2 to about 4 reversible bond/interaction moieties. In an embodiment, L is $(CH_2)_nCOOH$, with n=12-24, n=17-24, or n=16-18.

In an embodiment, L is $(CH_2)_nCOOH$, with n=1-16, e.g., n=2-8, e.g., n=4-6, e.g., n=3.

Embodiments of such building blocks include:

4-{4-[(Acetylamino-ethylcarbamoyl-methyl)-amino]-phenoxy}-N-dodecyl-butyramide;

4-(4-{[(3-Cyclopentyl-propionylamino)-ethylcarbamoyl-methyl]-amino}-phenoxy)-N-dodecyl-butyramide;

4-[4-({[2-(3-Chloro-phenyl)-acetylamino]-ethylcarbamoyl-methyl}-amino)-phenoxy]-N-dodecyl-butyramide;

N-{[4-(3-Dodecylcarbamoyl-propoxy)-phenylamino]-ethylcarbamoyl-methyl}-3-phenyl-acrylamide;

N-Dodecyl-4-(4-{[ethylcarbamoyl-(3-pyridin-3-yl-propionylamino)-methyl]-amino}-phenoxy)-butyramide;

N-Dodecyl-4-(4-{[ethylcarbamoyl-(2-methylsulfanyl-acetylamino)-methyl]-amino}-phenoxy)-butyramide;

N-{[4-(3-Dodecylcarbamoyl-propoxy)-phenylamino]-ethylcarbamoyl-methyl}-3-hydroxy-butyramide;

N-{[4-(3-Dodecylcarbamoyl-propoxy)-phenylamino]-ethylcarbamoyl-methyl}-succinamide;

4-(4-{[(4-Dimethylamino-butyrylamino)-ethylcarbamoyl-methyl]-amino}-phenoxy)-N-dodecyl-butyramide;

4-{4-[(Acetylamino-isobutylcarbamoyl-methyl)-amino]-phenoxy}-N-dodecyl-butyramide;

4-(4-{[(3-Cyclopentyl-propionylamino)-isobutylcarbamoyl-methyl]-amino}-phenoxy)-N-dodecyl-butyramide;

4-[4-({[2-(3-Chloro-phenyl)-acetylamino]-isobutylcarbamoyl-methyl}-amino)-phenoxy]-N-dodecyl-butyramide;

N-{[4-(3-Dodecylcarbamoyl-propoxy)-phenylamino]-isobutylcarbamoyl-methyl}-3-phenyl-acrylamide;

N-Dodecyl-4-(4-{[isobutylcarbamoyl-(3-pyridin-3-yl-propionylamino)-methyl]-amino}-phenoxy)-butyramide;

N-Dodecyl-4-(4-{[isobutylcarbamoyl-(2-methylsulfanyl-acetylamino)-methyl]-amino}-phenoxy)-butyramide;

N-{[4-(3-Dodecylcarbamoyl-propoxy)-phenylamino]-isobutylcarbamoyl-methyl}-3-hydroxy-butyramide;

N-{[3-(3-Dodecylcarbamoyl-propoxy)-phenylamino]-isobutylcarbamoyl-methyl}-succinamide;

4-(4-{[(4-Dimethylamino-butyrylamino)-isobutylcarbamoyl-methyl]-amino}-phenoxy)-N-dodecyl-butyramide;

4-{4-[(Acetylamino-phenethylcarbamoyl-methyl)-amino]-phenoxy}-N-dodecyl-butyramide;

4-(4-{[(3-Cyclopentyl-propionylamino)-phenethylcarbamoyl-methyl]-amino}-phenoxy)-N-dodecyl-butyramide;

4-(4-{[[2-(3-Chloro-phenyl)-acetylamino]-(3-methyl-hexa-3,5-dienylcarbamoyl)-methyl]-amino}-phenoxy)-N-dodecyl-butyramide;

N-{[4-(3-Dodecylcarbamoyl-propoxy)-phenylamino]-phenethylcarbamoyl-methyl}-3-phenyl-acrylamide;

N-Dodecyl-4-(4-{[phenethylcarbamoyl-(3-pyridin-3-yl-propionylamino)-methyl]-amino}-phenoxy)-butyramide;

N-Dodecyl-4-(4-{[(2-methylsulfanyl-acetylamino)-phenethylcarbamoyl-methyl]-amino}-phenoxy)-butyramide;

N-{[4-(3-Dodecylcarbamoyl-propoxy)-phenylamino]-phenethylcarbamoyl-methyl}-3-hydroxy-butyramide;

N-{[4-(3-Dodecylcarbamoyl-propoxy)-phenylamino]-phenethylcarbamoyl-methyl}-succinamide;

4-(4-{[(4-Dimethylamino-butyrylamino)-phenethylcarbamoyl-methyl]-amino}-phenoxy)-N-dodecyl-butyramide;

4-[4-({Acetylamino-[2-(4-methoxy-phenyl)-ethylcarbamoyl]-methyl}-amino)-phenoxy]-N-dodecyl-butyramide;

4-[4-({(3-Cyclopentyl-propionylamino)-[2-(4-methoxy-phenyl)-ethylcarbamoyl]-methyl}-amino)-phenoxy]-N-dodecyl-butyramide;

4-[4-({[2-(3-Chloro-phenyl)-acetylamino]-[2-(4-methoxy-phenyl)-ethylcarbamoyl]-methyl}-amino)-phenoxy]-N-dodecyl-butyramide;

N-{[4-(3-Dodecylcarbamoyl-propoxy)-phenylamino]-[2-(4-methoxy-phenyl)-ethylcarbamoyl]-methyl}-3-phenyl-acrylamide;

N-Dodecyl-4-(4-{[[2-(4-methoxy-phenyl)-ethylcarbamoyl]-(3-pyridin-3-yl-propionylamino)-methyl]-amino}-phenoxy)-butyramide;

N-Dodecyl-4-(4-{[[2-(4-methoxy-phenyl)-ethylcarbamoyl]-(2-methylsulfanyl-acetylamino)-methyl]-amino}-phenoxy)-butyramide;

N-{[4-(3-Dodecylcarbamoyl-propoxy)-phenylamino]-[2-(4-methoxy-phenyl)-ethylcarbamoyl]-methyl}-3-hydroxy-butyramide;

N-{[4-(3-Dodecylcarbamoyl-propoxy)-phenylamino]-[2-(4-methoxy-phenyl)-ethylcarbamoyl]-methyl}-succinamide;

4-[4-({(4-Dimethylamino-butyrylamino)-[2-(4-methoxy-phenyl)-ethylcarbamoyl]-methyl}-amino)-phenoxy]-N-dodecyl-butyramide;

4-(4-{[Acetylamino-(2-pyridin-2-yl-ethylcarbamoyl)-methyl]-amino}-phenoxy)-N-dodecyl-butyramide;

4-(4-{[(3-Cyclopentyl-propionylamino)-(2-pyridin-2-yl-ethylcarbamoyl)-methyl]-amino}-phenoxy)-N-dodecyl-butyramide;

4-(4-{[[2-(3-Chloro-phenyl)-acetylamino]-(2-pyridin-2-yl-ethylcarbamoyl)-methyl]-amino}-phenoxy)-N-dodecyl-butyramide;

N-[[4-(3-Dodecylcarbamoyl-propoxy)-phenylamino]-(2-pyridin-2-yl-ethylcarbamoyl)-methyl]-3-phenyl-acrylamide;

N-Dodecyl-4-(4-{[(2-pyridin-2-yl-ethylcarbamoyl)-(3-pyridin-3-yl-propionylamino)-methyl]-amino}-phenoxy)-butyramide

N-Dodecyl-4-(4-{[(2-methylsulfanyl-acetylamino)-(2-pyridin-2-yl-ethylcarbamoyl)-methyl]-amino}-phenoxy)-butyramide;

N-[[4-(3-Dodecylcarbamoyl-propoxy)-phenylamino]-(2-pyridin-2-yl-ethylcarbamoyl)-methyl]-3-hydroxy-butyramide;

N-[[4-(3-Dodecylcarbamoyl-propoxy)-phenylamino]-(2-pyridin-2-yl-ethylcarbamoyl)-methyl]-succinamide;

4-(4-{[(4-Dimethylamino-butyrylamino)-(2-pyridin-2-yl-ethylcarbamoyl)-methyl]-amino}-phenoxy)-N-dodecyl-butyramide;

4-(4-{[Acetylamino-(2-methoxy-ethylcarbamoyl)-methyl]-amino}-phenoxy)-N-dodecyl-butyramide;

4-(4-{[(3-Cyclopentyl-propionylamino)-(2-methoxy-ethylcarbamoyl)-methyl]-amino}-phenoxy)-N-dodecyl-butyramide;

4-(4-{[[2-(3-Chloro-phenyl)-acetylamino]-(2-methoxy-ethylcarbamoyl)-methyl]-amino}-phenoxy)-N-dodecyl-butyramide;

N-[[4-(3-Dodecylcarbamoyl-propoxy)-phenylamino]-(2-methoxy-ethylcarbamoyl)-methyl]-3-phenyl-acrylamide;

N-Dodecyl-4-(4-{[(2-methoxy-ethylcarbamoyl)-(3-pyridin-3-yl-propionylamino)-methyl]-amino}-phenoxy)-butyramide;

N-Dodecyl-4-(4-{[(2-methoxy-ethylcarbamoyl)-(2-methylsulfanyl-acetylamino)-methyl]-amino}-phenoxy)-butyramide;

N-[[4-(3-Dodecylcarbamoyl-propoxy)-phenylamino]-(2-methoxy-ethylcarbamoyl)-methyl]-3-hydroxy-butyramide;

N-[[3-(3-Dodecylcarbamoyl-propoxy)-phenylamino]-(2-methoxy-ethylcarbamoyl)-methyl]-succinamide;

4-(4-{[(4-Dimethylamino-butyrylamino)-(2-methoxy-ethylcarbamoyl)-methyl]-amino}-phenoxy)-N-dodecyl-butyramide;

4-(4-{[Acetylamino-(2-hydroxy-ethylcarbamoyl)-methyl]-amino}-phenoxy)-N-dodecyl-butyramide;

4-(4-{[(3-Cyclopentyl-propionylamino)-(2-hydroxy-ethylcarbamoyl)-methyl]-amino}-phenoxy)-N-dodecyl-butyramide;

4-(4-{[[2-(3-Chloro-phenyl)-acetylamino]-(2-hydroxy-ethylcarbamoyl)-methyl]-amino}-phenoxy)-N-dodecyl-butyramide;

N-[[4-(3-Dodecylcarbamoyl-propoxy)-phenylamino]-(2-hydroxy-ethylcarbamoyl)-methyl]-3-phenyl-acrylamide;

N-Dodecyl-4-(4-{[(2-hydroxy-ethylcarbamoyl)-(3-pyridin-3-yl-propionylamino)-methyl]-amino}-phenoxy)-butyramide;

N-Dodecyl-4-(4-{[(2-hydroxy-ethylcarbamoyl)-(2-methylsulfanyl-acetylamino)-methyl]-amino}-phenoxy)-butyramide;

N-[[4-(3-Dodecylcarbamoyl-propoxy)-phenylamino]-(2-hydroxy-ethylcarbamoyl)-methyl]-3-hydroxy-butyramide;

N-[[3-(3-Dodecylcarbamoyl-propoxy)-phenylamino]-(2-hydroxy-ethylcarbamoyl)-methyl]-succinamide;

4-(4-{[(4-Dimethylamino-butyrylamino)-(2-hydroxy-ethylcarbamoyl)-methyl]-amino}-phenoxy)-N-dodecyl-butyramide;

4-(4-{[Acetylamino-(2-acetylamino-ethylcarbamoyl)-methyl]-amino}-phenoxy)-N-dodecyl-butyramide;

4-(4-{[(2-Acetylamino-ethylcarbamoyl)-(3-cyclopentyl-propionylamino)-methyl]-amino}-phenoxy)-N-dodecyl-butyramide;

4-[4-({(2-Acetylamino-ethylcarbamoyl)-[2-(3-chloro-phenyl)-acetylamino]-methyl}-amino)-phenoxy]-N-dodecyl-butyramide;

N-{(2-Acetylamino-ethylcarbamoyl)-[4-(3-dodecylcarbamoyl-propoxy)-phenylamino]-methyl}-3-phenyl-acrylamide;

4-(4-{[(2-Acetylamino-ethylcarbamoyl)-(3-pyridin-3-yl-propionylamino)-methyl]-amino}-phenoxy)-N-dodecyl-butyramide;

4-(4-{[(2-Acetylamino-ethylcarbamoyl)-(2-methylsulfanyl-acetylamino)-methyl]-amino}-phenoxy)-N-dodecyl-butyramide;

N-{(2-Acetylamino-ethylcarbamoyl)-[4-(3-dodecylcarbamoyl-propoxy)-phenylamino]-methyl}-3-hydroxy-butyramide;

N-{(2-Acetylamino-ethylcarbamoyl)-[3-(3-dodecylcarbamoyl-propoxy)-phenylamino]-methyl}-succinamide;

4-(4-{[(2-Acetylamino-ethylcarbamoyl)-(4-dimethylamino-butyrylamino)-methyl]-amino}-phenoxy)-N-dodecyl-butyramide;

4-(4-{[Acetylamino-(2-pyrrolidin-1-yl-ethylcarbamoyl)-methyl]-amino}-phenoxy)-N-dodecyl-butyramide;

4-(4-{[(3-Cyclopentyl-propionylamino)-(2-pyrrolidin-1-yl-ethylcarbamoyl)-methyl]-amino}-phenoxy)-N-dodecyl-butyramide;

4-(4-{[[2-(3-Chloro-phenyl)-acetylamino]-(2-pyrrolidin-1-yl-ethylcarbamoyl)-methyl]-amino}-phenoxy)-N-dodecyl-butyramide;

N-[[4-(3-Dodecylcarbamoyl-propoxy)-phenylamino]-(2-pyrrolidin-1-yl-ethylcarbamoyl)-methyl]-3-phenyl-acrylamide;

N-Dodecyl-4-(4-{[(3-pyridin-3-yl-propionylamino)-(2-pyrrolidin-1-yl-ethylcarbamoyl)-methyl]-amino}-phenoxy)-butyramide;

N-Dodecyl-4-(4-{[(2-methylsulfanyl-acetylamino)-(2-pyrrolidin-1-yl-ethylcarbamoyl)-methyl]-amino}-phenoxy)-butyramide;

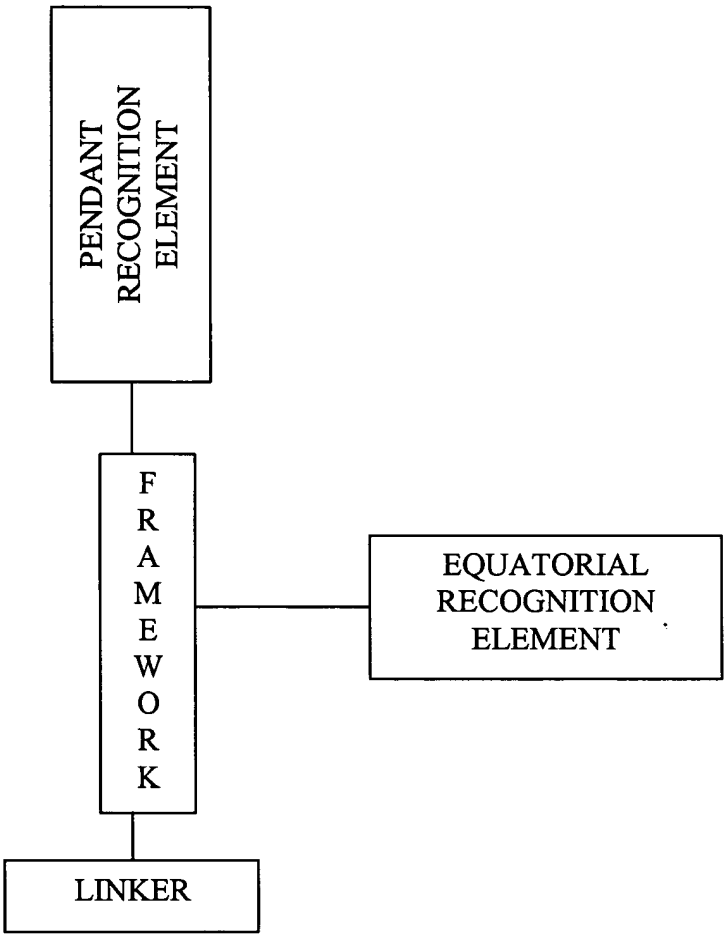
N-[[4-(3-Dodecylcarbamoyl-propoxy)-phenylamino]-(2-pyrrolidin-1-yl-ethylcarbamoyl)-methyl]-3-hydroxy-butyramide;

N-[[3-(3-Dodecylcarbamoyl-propoxy)-phenylamino]-(2-pyrrolidin-1-yl-ethylcarbamoyl)-methyl]-succinamide;

4-(4-{[(4-Dimethylamino-butyrylamino)-(2-pyrrolidin-1-yl-ethylcarbamoyl)-methyl]-amino}-phenoxy)-N-dodecyl-butyramide;

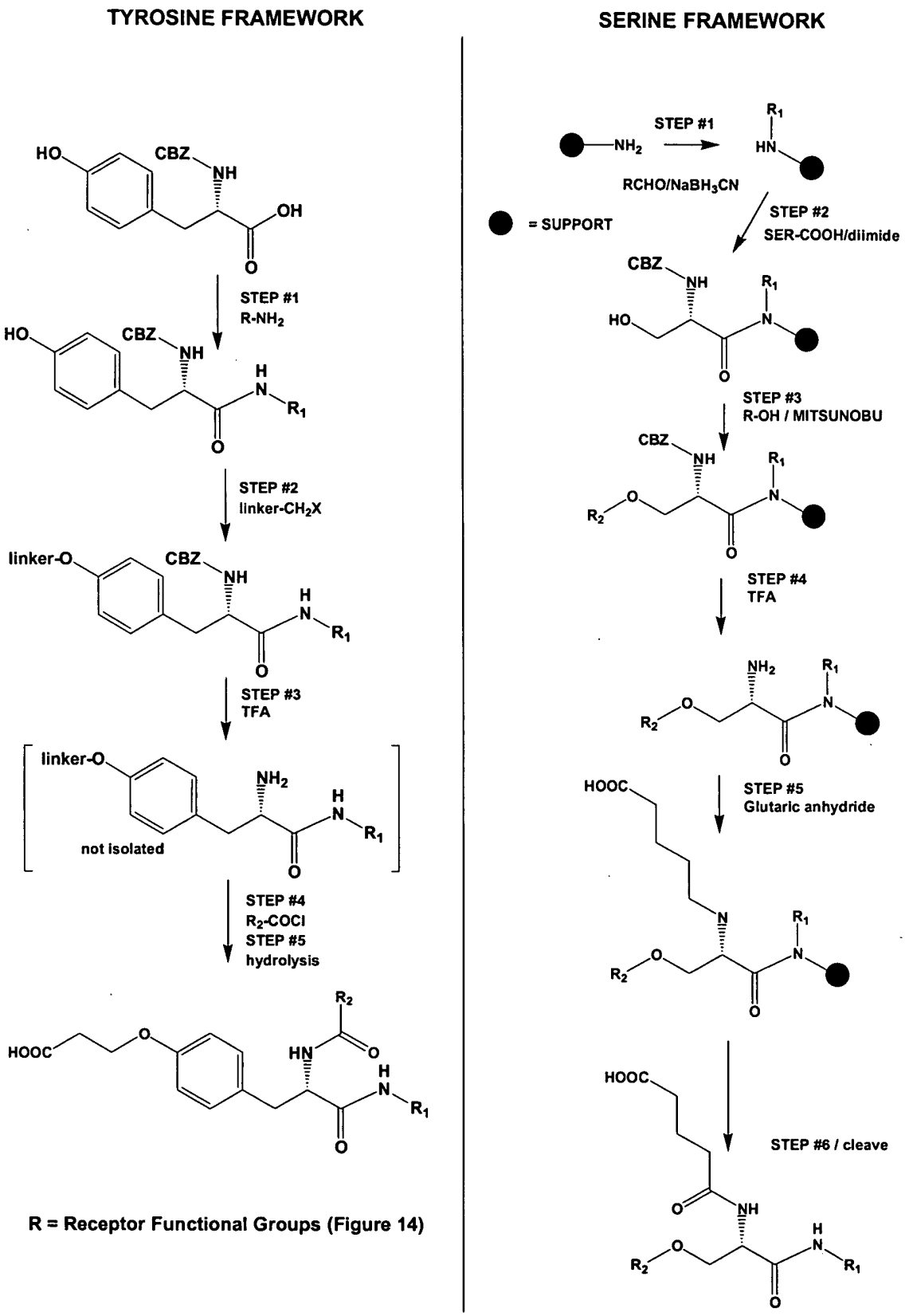
salts thereof, esters thereof, protected or blocked derivatives thereof, immobilized derivatives thereof, derivatives thereof, or mixtures thereof. The nomenclature in this paragraph is according to the program CS CHEMDRAW ULTRA®.

Building blocks of Formula 2 and including an A recognition element, a B recognition element, a linker, and a framework of a naturally occurring α-amino acid can be visualized as having the B recognition element in an equatorial configuration and the A recognition element in a pendant configuration. An embodiment of such a configuration is schematically illustrated in Scheme 3:



Scheme 3

Building blocks including an A and/or a B recognition element, a linker, and an amino acid framework can be made by methods illustrated in general Scheme 4.


TYROSINE FRAMEWORK
SERINE FRAMEWORK
HO
CBZ
NH
OH
O
STEP #1
R-NH2
H
N
R1
STEP #2
linker-CH2X
linker-O
STEP #3
TFA
NH2
not isolated
STEP #4
R2-COCl
STEP #5
hydrolysis
R2
HOOC
HN
R = Receptor Functional Groups (Figure 14)
STEP #1
RCHO/NaBH3CN
= SUPPORT
STEP #2
SER-COOH/diimide
STEP #3
R-OH / MITSUNOBU
STEP #4
TFA
STEP #5
Glutaric anhydride
STEP #6 / cleave

Scheme 4

Embodiments of Building Blocks Reversibly Immobilized on Lawn or Support

The present invention includes building blocks reversibly immobilized on a lawn or a support through any of a variety of interactions or combination of interactions described above. In an embodiment, the functionalized lawn includes a first covalent bonding moiety and the building block includes a second covalent bonding moiety. The first and second covalent bonding moieties can form or can be coupled by a readily reversible covalent bond. In an embodiment, the first covalent bonding moiety includes an amine nitrogen and the second covalent bonding moiety includes a carbonyl carbon. In an embodiment, the first covalent bonding moiety includes a carbonyl carbon and the second covalent bonding moiety includes an amine nitrogen.

In an embodiment, the first covalent bonding moiety includes an amine nitrogen and the second covalent bonding moiety includes a carbonyl carbon; the first covalent bonding moiety includes a carbonyl carbon and the second covalent bonding moiety includes an amine nitrogen; or a mixture or a combination thereof.

In an embodiment, the functionalized lawn includes a first charged moiety and the building block includes a second charged moiety. In such an embodiment, the first and second charged moieties advantageously have opposite charges. In an embodiment, the first charged moiety includes a carboxylate and the second charged moiety includes an ammonium. In an embodiment, the first charged moiety includes an ammonium and the second charged moiety includes a carboxylate.

In an embodiment, the first charged moiety includes a carboxylate and the second charged moiety includes an ammonium; the first charged moiety includes an ammonium and the second charged moiety includes a carboxylate; or a mixture or a combination thereof.

In an embodiment, the functionalized lawn includes a first lipophilic moiety and the building block includes a second lipophilic moiety. In an embodiment, the first and second lipophilic moieties includes independently one or more branched or straight chain C_{6-36} alkyl, C_{8-24} alkyl, C_{12-24} alkyl, C_{12-18} alkyl, or the like; C_{6-36} alkenyl, C_{8-24} alkenyl, C_{12-24} alkenyl, C_{12-18} alkenyl, or the like, with, for example, 1 to 4 double bonds; C_{6-36} alkynyl, C_{8-24} alkynyl, C_{12-24} alkynyl, C_{12-18} alkynyl, or the like, with, for example, 1 to 4 triple bonds; chains with 1-4 double or triple bonds; chains including aryl or substituted aryl moieties (e.g., phenyl or naphthyl moieties at the end or middle of a chain); polyaromatic hydrocarbon moieties;

cycloalkane or substituted alkane moieties with numbers of carbons as described for chains; combinations or mixtures thereof; or the like. The alkyl, alkenyl, or alkynyl group can include branching; within chain functionality like an ether group; terminal functionality like alcohol, amide, carboxylate or the like; or the like.

In an embodiment, the functionalized lawn includes a first lipophilic moiety and a first covalent bonding moiety; and the building block includes a second lipophilic moiety and a second covalent bonding moiety. In an embodiment, the functionalized lawn includes a first lipophilic moiety and a first charged moiety; and the building block includes a second lipophilic moiety and a second charged moiety. In an embodiment, the functionalized lawn includes a first lipophilic moiety and a first covalent bonding moiety and the building block includes a second lipophilic moiety and a second covalent bonding moiety; the functionalized lawn includes a first lipophilic moiety and a first charged moiety; and the building block includes a second lipophilic moiety and a second charged moiety; or a combination or a combination thereof.

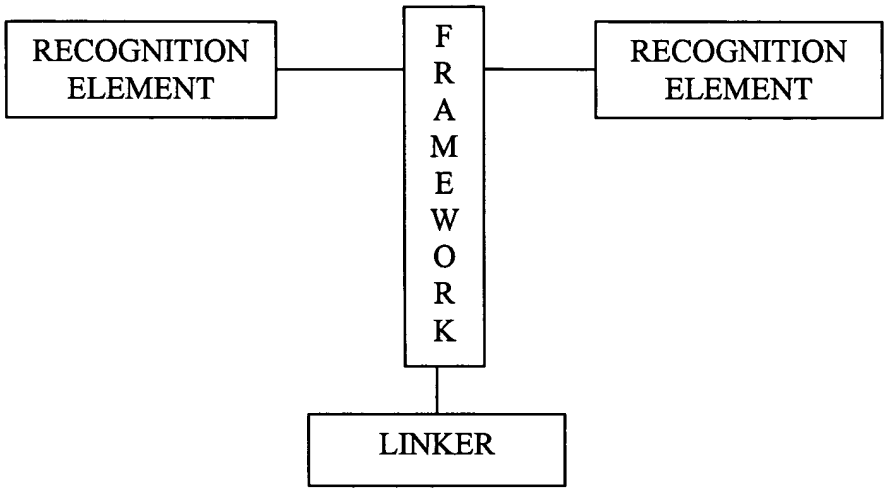
In an embodiment, the present invention includes a heterogeneous building block array. Such a building block array can include a support, a functionalized lawn, and a plurality of building blocks. The functionalized lawn can be coupled to the support. The array can include a plurality of regions on the support, and the regions can include a plurality of building blocks. In this embodiment, the plurality of building blocks can be reversibly immobilized on the lawn.

In an embodiment, the present invention includes a composition. Such a composition can include a support, a functionalized lawn, and a plurality of building blocks. The functionalized lawn can be coupled to the surface, and a region on the surface can include a plurality of building blocks. In this embodiment, the building blocks can be reversibly immobilized on the lawn.

More on Building Blocks

Building blocks can be asymmetric. Employing asymmetry, various combinations of, for example, linker and recognition elements can produce building blocks that can be visualized to occupy 3D space in different ways. As a consequence, these different building blocks can perform binding related but otherwise distinct functions.

In an embodiment, building blocks including two recognition elements, a linker, and a framework can be visualized as having both recognition elements in spreading pendant configurations. An embodiment of such a configuration is schematically illustrated in Scheme 5:



Scheme 5

Such a configuration has a molecular footprint with substantial area in two dimensions. Such a larger footprint can be suitable, for example, for binding larger ligands that prefer or require interactions with a receptor over a larger area or that prefer or require interactions with a larger number of functional groups on the recognition element. Such larger ligands can include proteins, carbohydrates, cells, and microorganisms (e.g., bacteria and viruses).

In an embodiment, a building block can have only a single recognition element in a pendant configuration and a pendant linker distal on the framework. Such building blocks can be compact. Such a building block can interact with large molecules that include a binding region, such as a protein (e.g., enzyme or receptor) or other macromolecule. For example, such a building block can be employed to probe cavities, such as binding sites, on proteins.

Sets of Building Blocks

Embodiments of Sets or Kits of Reagents

The present invention includes compositions, articles of manufacture, kits, and reagents that can make, form, or include artificial receptors, such as candidate artificial receptors. Such an artificial receptor can include or be part of a dynamic building block array.

In an embodiment, the present invention includes an article of manufacture. Such an article of manufacture can include a support, a functionalized lawn reagent, and a plurality of building blocks. The functionalized lawn can be configured to be coupled to the support. The plurality of building blocks can be configured to be reversibly coupled to the lawn. For example, the functionalized lawn reagent can include a first covalent bonding moiety and the building block comprises a second covalent bonding moiety. For example, the functionalized lawn reagent can include a first charged moiety and the building block comprises a second charged moiety, the first and second charged moieties having opposite charges. For example, the functionalized lawn reagent can include a first lipophilic moiety and the building block comprises a second lipophilic moiety. The article of manufacture can include a functionalized glass support.

Embodiments of Sets of Building Blocks

The present invention also relates to sets of building blocks. The sets of building blocks can include isolated building blocks, building blocks with an activated linker for coupling to a support, and/or building blocks coupled to a support. Sets of building blocks include a plurality of building blocks. The plurality of building blocks can be a component of a coating, of a spot or spots (e.g., forming candidate artificial receptor(s)), or of a kit. The plurality of building blocks can include a sufficient number of building blocks and recognition elements for exploring candidate artificial receptors or for defining receptors for a ligand. That is, the set of building blocks can include a majority (e.g., at least 6) of the structural characteristics selected from positive charge, negative charge, acid, base, electron acceptor, electron donor, hydrogen bond donor, hydrogen bond acceptor, free electron pair, π electrons, charge polarization, hydrophilicity, hydrophobicity.

For a set of building blocks, the recognition elements can be selected to provide a variety of structural characteristics to the individual members of the set. A single building block can include recognition elements with more than one of the structural characteristics. A set of building blocks can include recognition elements with each of the structural characteristics. For example, a set of building blocks can include one or more building blocks including a positively charged recognition element, one or more building blocks including a negatively charged recognition element, one or more building blocks including an acidic recognition element, one or more building blocks including a basic recognition element, one or more building blocks including an electron donating recognition element, one or more building blocks including an electron accepting recognition element, one or more building blocks including a hydrogen bond donor recognition element, one or more building blocks including a hydrogen bond acceptor recognition element, one or more building blocks including a polar recognition element, one or more building blocks including a recognition element with free electron pair(s), one or more building blocks including a recognition element with π electrons, one or more building blocks including a hydrophilic recognition element, one or more building blocks including a hydrophobic recognition element, one or more building blocks including a small recognition element, and/or one or more building blocks including a bulky recognition element.

In an embodiment, the number and variety of recognition elements is selected to provide a set of building blocks with a manageable number of members. A manageable number of building blocks provides, for example, fewer than 10 million combinations, e.g., about 2 million combinations, with each combination including, for example, 3, 4, 5, or 6 building blocks. In an embodiment, the recognition elements provide a set of building blocks that incorporate the functional groups and configurations found in the components of natural receptors, in an embodiment, with the smallest number of building blocks.

The nine A and nine B recognition elements can be incorporated into a set of 81 (9x9) building blocks, each with one A and one B recognition element. Such building blocks can, for example, be prepared using combinatorial syntheses on a framework, such as a serine or tyrosine framework. In groups of 4, this set of 81 building blocks provides 1.66 million combinations of building blocks (Table 1), each of which can be a heterogeneous combination in a microarray on a support, substrate, or scaffold. Although not limiting to the

present invention, it is believed that these groups of 4 are sufficient to incorporate the functional groups and configurations found in natural receptors and to provide sufficient candidate artificial receptors to yield one or more artificial receptors for a specified ligand.

Table 1 - - Calculation of the Number of Candidate Artificial Receptor Combinations

Discrete combinations calculated using the following formula for N compounds taken in groups of n (CRC Standard Math Tables and Formulas Handbook, 30th ed.):

$$\text{Number of Combinations} = N! / (N-n)!\, n!$$

For N = 81

GROUP	COMBINATIONS
n = 1	81
n = 2	3,240
n = 3	85,320
n = 4	1,663,740

A set of building blocks can include building blocks of general Formula 2, with RE_1 being B1, B2, B3, B3a, B4, B5, B6, B7, B8, or B9 and with RE_2 being A1, A2, A3, A3a, A4, A5, A6, A7, A8, or A9. In an embodiment of the set, RE_1 can be B2, B4, or B6 and RE_2 can be A2, A4, or A6. In an embodiment of the set, RE_1 can be B1, B3, B3a, B6, or B8 and RE_2 can be A2, A4, A5, or A9. In an embodiment of the set, RE_1 can be B2, B4, B6, or B8 and RE_2 can be A2, A4, A6, or A8. In an embodiment of the set, RE_1 can be B1, B2, B4, B6, or B8 and RE_2 can be A1, A2, A4, A6, or A8. In an embodiment of the set, RE_1 can be B1, B2, B4, B6, B8, or B9 and RE_2 can be A1, A2, A4, A6, A8, or A9. In an embodiment of the set, RE_1 can be B1, B2, B3, B3a, B4, B5, B6, B7, B8, or B9 and RE_2 can be A1, A2, A3, A3a, A4, A5, A6, A7, A8, or A9.

In an embodiment, a set of building blocks includes alkyl, aryl, and polar recognition elements, plus recognition elements that are combinations of these structural characteristics. A set of building blocks including those of general Formula 2, with RE_1 being B1, B2, B3, B3a, B4, B5, B6, B7, B8, or B9 and with RE_2 being A1, A2, A3, A3a, A4, A5, A6, A7, A8, or A9 is a set of building blocks with includes alkyl, aryl, and polar recognition elements. Table 2 illustrates an embodiment of 81 building blocks of general Formula 2 with recognition elements that span alkyl, aryl, and polar recognition elements.

Figures 8, 9A, and 9B illustrate plots of volume versus logP for building blocks including each of the 9 A and 9 B recognition elements and artificial receptors made from these building blocks. These plots illustrate that the building blocks with A and B recognition elements and artificial receptors made from these building blocks fill a significant portion of the binding space defined by volume and lipophilicity/hydrophilicity.

Table 2 - Embodiment of 81 Building Blocks of General Formula 2 with Recognition Elements that Span Alkyl, Aryl, and Polar Recognition Elements.

RE_1, EQUATORIAL

RE1 / RE2	B1	B2	B3	B4	B5	B6	B7	B8	B9
A1	A1-B1	A1-B2	A1-B3	A1-B4	A1-B5	A1-B6	A1-B7	A1-B8	A1-B9
A2	A2-B1	A2-B2	A2-B3	A2-B4	A2-B5	A2-B6	A2-B7	A2-B8	A2-B9
A3	A3-B1	A3-B2	A3-B3	A3-B4	A3-B5	A3-B6	A3-B7	A3-B8	A3-B9
A4	A4-B1	A4-B2	A4-B3	A4-B4	A4-B5	A4-B6	A4-B7	A4-B8	A4-B9
A5	A5-B1	A5-B2	A5-B3	A5-B4	A5-B5	A5-B6	A5-B7	A5-B8	A5-B9
A6	A6-B1	A6-B2	A6-B3	A6-B4	A6-B5	A6-B6	A6-B7	A6-B8	A6-B9
A7	A7-B1	A7-B2	A7-B3	A7-B4	A7-B5	A7-B6	A7-B7	A7-B8	A7-B9
A8	A8-B1	A8-B2	A8-B3	A8-B4	A8-B5	A8-B6	A8-B7	A8-B8	A8-B9
A9	A9-B1	A9-B2	A9-B3	A9-B4	A9-B5	A9-B6	A9-B7	A9-B8	A9-B9

RE_2, PENDANT

Embodiments of Sets of Building Blocks

The present invention includes sets of building blocks. Sets of building blocks can include 2 or more building blocks coupled to a support or scaffold. Such a support or scaffold can be referred to as including heterogeneous building blocks. As used herein, the term "support" refers to a solid support that is, for example, macroscopic. As used herein, the term scaffold refers to a molecular scale structure to which a plurality of building blocks

can covalently bind. The two or more building blocks can be coupled to the support or scaffold in a molecular configuration with different building blocks in proximity to one another. Such a molecular configuration of a plurality of different building blocks provides a candidate artificial receptor.

Building Blocks on Supports

The present invention includes immobilized sets and combinations of building blocks. In an embodiment, the present invention includes a solid support having on its surface a plurality of building blocks.

For example, the support can be a glass tube or well coated with a plurality of building blocks. In an embodiment, the surface of the glass tube or well (e.g., a 96 well plate) coated with a coating to which the plurality of building blocks are covalently bound. Such a coating can be referred to as including heterogeneous building blocks. The surface or coating can include a density of building blocks sufficient to provide interactions of more than one building block with a ligand. The building blocks can be in proximity to one another. Evidence of proximity of different building blocks is provided by altered (e.g., tighter or looser) binding of a ligand to a surface with a plurality of building blocks compared to a surface with only one of the building blocks.

A set of building blocks can be employed in combinations of 2, 3, 4, or more building blocks on an individual tube or well. For this embodiment, with each combination using a bulky tube or well, a manageable set of building blocks can provide fewer than several hundred or several thousand combinations of building blocks. For example, in this context, a set of 3, 4, 5, or 6 building blocks provides a manageable number of combinations of 2, 3, or 4 building blocks.

In an embodiment, immobilized combinations of building blocks can include a plurality of tubes each tube having immobilized on its surface a heterogeneous combination of building blocks. The building blocks can be immobilized on the surface of the tube through amide links between each building block and a support matrix. The immobilized building blocks can include combinations of 2, 3, or 4 building blocks. For convenience in limiting the number of tubes handled, in this embodiment a set includes up to 5-7 building blocks, e.g., 5 or fewer, e.g., 3, 4, or 5. For tubes, suitable building blocks have general

Formula 2, with RE_1 being B1, B2, B3, B3a, B4, B5, B6, B7, B8, or B9 and with RE_2 being A1, A2, A3, A3a, A4, A5, A6, A7, A8, or A9. In an embodiment for tubes, RE_1 can be B1, B3, B3a, B6, or B8 and RE_2 can be A2, A4, A5, or A9. In an embodiment for tubes, RE_1 can be B2, B4, or B6 and RE_2 can be A2, A4, or A6. In an embodiment for tubes, RE_1 can be B2, B4, B6, or B8 and RE_2 can be A2, A4, A6, or A8. In an embodiment for tubes, RE_1 can be B1, B2, B4, B6, or B8 and RE_2 can be A1, A2, A4, A6, or A8. In an embodiment for tubes, RE_1 can be B1, B2, B4, B6, B8, or B9 and RE_2 can be A1, A2, A4, A6, A8, or A9. A plurality of tubes each coated with a combination of building blocks can be configured as an array of tubes.

In an embodiment, the present invention includes a solid support having on its surface a plurality of regions or spots, each region or spot including a plurality of building blocks. For example, the support can be a glass slide spotted with a plurality of spots, each spot including a plurality of building blocks. Such a spot or region can be referred to as including heterogeneous building blocks. Each region or spot can include a density of building blocks sufficient to provide interactions of more than one building block with a ligand. Although each region or spot can be separated from the others, in the region or spot, the building blocks can be in proximity to one another. Evidence of proximity of different building blocks in a region or spot is provided by altered (e.g., tighter or looser) binding of a ligand to a surface with a plurality of building blocks compared to a region or spot with only one of the building blocks. A plurality of regions or spots of building blocks is referred to herein as an array of regions or spots.

A set of building blocks can be employed in combinations of 2, 3, 4, or more building blocks in each region or spot. In such an embodiment, up to 100,000 spots can fit on a glass slide. Therefore, a manageable set of building blocks can provide several million combinations of building blocks. For example, in this context, a set of 81 building blocks provides a manageable number of (1.66 million) combinations of 4 building blocks. Although not limiting to the present invention, it is believed that these 1.66 million combinations are sufficient to incorporate the functional groups and configurations found in natural receptors and to provide sufficient candidate artificial receptors to yield one or more artificial receptors for a specified ligand.

In an embodiment, immobilized combinations of building blocks can include one or more glass slides, each slide having on its surface a plurality of spots, each spot including an immobilized heterogeneous combination of building blocks. The building blocks can be immobilized on the surface of the slide through amide links between each building block and a support matrix. The immobilized building blocks can include, for example, combinations of 2, 3, 4, 5, or 6 building blocks.

For convenience in limiting the number of slides handled, in this embodiment a set includes up to 200 building blocks, e.g., 50-100, e.g., about 80 (including 81) building blocks. For slides, suitable building blocks have general Formula 2, with RE_1 being B1, B2, B3, B3a, B4, B5, B6, B7, B8, or B9 and with RE_2 being A1, A2, A3, A3a, A4, A5, A6, A7, A8, or A9. This embodiment can include a group of slides with 1.7 million heterogeneous spots, each spot including 4 building blocks.

In an embodiment, the one or more slides can include heterogeneous spots of building blocks made from combinations of a subset of the total building blocks and/or smaller groups of the building blocks in each spot. That is, each spot includes only, for example, 2 or 3 building blocks, rather than 4 or 5. For example, the one or more slides can include the number of spots formed by combinations of a full set of building blocks (e.g. 81 of a set of 81) in groups of 2 and/or 3. For example, the one or more slides can include the number of spots formed by combinations of a subset of the building blocks (e.g., 25 of the set of 81) in groups of 4 or 5. For example, the one or more slides can include the number of spots formed by combinations of a subset of the building blocks (e.g., 25 of the set of 81) in groups of 2 or 3. Should a candidate artificial receptor of interest be identified from the subset and/or smaller groups, then additional subsets and groups can be made or selected incorporating the building blocks in the candidates of interest or structurally similar building blocks.

For example, Figure 11 illustrates that a single slide with the 3,240 n=2 derived combinations can be used to define a more limited set from the 81 building blocks. This defined set of e.g. 25 (defined from a 5x5 matrix of the n=2 results) can be used to produce an additional 2,300 n=3 derived and 12,650 n=4 derived combinations which can be probed to define the optimum receptor configuration. Further optimization can be pursued using

ratios of the best building blocks which deviate from 1:1 followed by specific synthesis of the identified receptor(s).

Building blocks can be coupled to supports using known methods for activating compounds of the types employed as building blocks and for coupling them to supports. For example, building blocks including activated esters can be coupled to supports including amine functional groups. A carboxyl group on a building block can be derivatized to form the activated ester. By way of further example, building blocks including amine functional groups can be coupled to supports including carboxyl groups. Pairs of functional groups that can be employed on building blocks and supports include amine and carboxyl (or activated carboxyl), thiol and maleimide, and the like.

Individual or combinations of building blocks can be coupled to the supports in spots using conventional micro spotting techniques (e.g., piezoelectric, pin, and electromagnetic printers). Such spotting yields a microarray of spots of heterogeneous combinations of building blocks, each of which can be a candidate artificial receptor. As described herein above, each spot in a microarray includes a statistically significant number of each building block.

The set of building blocks can be on any of the variety of known supports employed in combinatorial or synthetic chemistry (e.g., a microscope slide, a bead, a resin, a gel, or the like). Suitable supports include functionalized glass, such as a functionalized slide or tube, glass microscope slide, glass plate, glass coverslip, glass beads, microporous glass beads, silica gel supports, and the like. As described hereinabove, a glass support can include a support matrix of silanating agent with functional groups suitable for coupling to a building block. For use in sets of building blocks, the support matrix functional groups can be pendant from the support in groups of one (e.g., as a lawn of amines or another functional group) or in groups of, for example, 2, 3, 4, 5, 6, or 7. The groups of a plurality of functional groups pendant from the support can be visualized as scaffold molecules pendant from the support.

The surface of the support can be visualized as including a floor and the building blocks (Figures 3A, 3B, and 4). Thus, the floor can be considered a feature of the candidate artificial receptor. In an embodiment, the candidate artificial receptor can include building blocks and unmodified amines of the floor. Such a candidate artificial receptor has an

amine/ammonium floor. In an embodiment, the candidate artificial receptor can include building blocks and modified amines of the floor (e.g., the acetamide).

Sets on Scaffolds

In an embodiment, the present invention includes a scaffold molecule having coupled to it a plurality of building blocks. For example, the scaffold can be a polyamine, for example, a cyclic molecule with a plurality of primary amine groups around the ring. Such a scaffold can include a plurality of building blocks coupled to the amines. Such a scaffold can be referred to as including heterogeneous building blocks. The scaffold can provide a density of building blocks sufficient to provide interactions of more than one building block with a ligand. The building blocks can be in proximity to one another. Evidence of proximity of different building blocks on a scaffold is provided by altered (e.g., tighter or looser) binding of a ligand to a scaffold with a plurality of building blocks compared to the scaffold with only one of the building blocks. The scaffold can be coupled to a support. Scaffolds can include functional groups for coupling to, for example, 2, 3, 4, 5, 6, or 7 building blocks.

A scaffold can be the support for an artificial receptor including a combination of 3, 4, or more building blocks occupying distinct positions relative to one another on the scaffold. For example, building block 1 can be adjacent to any of building blocks 2, 3, or 4. This can be illustrated by considering the building blocks coupled to different functional groups on a scaffold. For example, Figure 12 illustrates positional isomers of 4 different building blocks at the vertices of a quadrilateral shaped scaffold. Scaffold positional isomer artificial receptors can be made, for example, on a scaffold with multiple functional groups that can be protected and deprotected by orthogonal chemistries.

Such a scaffold positional isomer artificial receptor can provide a lead or working receptor with utility distinct from a solid support based receptor. For example, such a scaffold positional isomer can be evaluated and selected for optimal binding, then employed where an optimal receptor is required. The scaffold artificial receptor can be immobilized, for example, on a light fiber to provide a detectable signal or for any of the other applications described herein for working artificial receptors.

A scaffold artificial receptor that has not been immobilized can be used in applications in which an antibody can be used, as a specific anticancer agent, to bind and immobilize/neutralize bloodstream components like cholesterol, cocaine or DDT, to bind and neutralize hazardous wastes, in the development of free solution analysis methods, e.g. fluorescence polarization immunoassay or molecular beacon based assays. Such free (not immobilized) scaffold artificial receptors can also be used for development of pharmaceuticals based on binding, e.g. application of scaffold receptors to block protein-protein interactions which are involved in cancer, the progression of AIDS, the development of tuberculoses and malaria, the toxic effects produced by exposure to industrial chlorinated aromatics, and the like.

In an embodiment, the scaffold artificial receptor is introduced into a subject (e.g., mouse, rat, dog, cat, horse, monkey, human, or the like) through, for example, injection, ingestion, gavage, suppository, inhalation, or the like. Once introduced, the scaffold artificial receptor can bind a compound of interest, such as cocaine, cholesterol, lead, DDT. Binding of the scaffold artificial receptor binding can target the bound material for detection, destruction, excretion, therapy, or the like.

In an embodiment, the scaffold artificial receptor is contacted with an environmental matrix (e.g., water, soil, sediment) through, for example, mixing, spraying, injection, or the like. In the matrix, the scaffold artificial receptor binds a ligand of interest. For a ligand of interest that is a hazardous waste component, a hazardous waste mixture, a pollution component, a pollution mixture, or the like, binding to the scaffold artificial receptor can target the bound material for detection, destruction, or immobilization.

In an embodiment, the scaffold artificial receptor is to a conjugated biological effector. Such a biological effector can be a toxin, a radioisotope chelate, or the like. The conjugate can be introduced into a subject. After introduction, the scaffold artificial receptor conjugate can interact with a ligand of interest that is associated with, for example, a disease causing microbe or a cancer cell. This interaction targets the conjugated toxin or radioisotope chelate to the disease causing microbe or cancer cell for the detection, therapy, destruction of the infectious agent or cancerous cell.

In an embodiment, the scaffold artificial receptor is used in free solution analysis methods. For example a scaffold artificial receptor can include a fluorophore or molecular

beacon. Binding of the scaffold artificial receptor conjugate to a ligand of interest or a sample containing a ligand of interest then produces fluorescence polarization or molecular beacon recombination which produces a signal which is related to the presence of the ligand of interest.

In an embodiment, the scaffold artificial receptor can be used as a pharmaceutical, for example, for the treatment of cancer, infection, disease, or toxic effects. As a pharmaceutical, binding of the scaffold artificial receptor to a ligand of interest (e.g., on or in a cell or microbe) can block, for example, DNA replication, gene regulation, RNA transcription, peptide synthesis. Such blocking can disrupt protein (e.g., enzyme) synthesis or modification, protein-protein interactions or the like. Such synthesis, modification, or interactions can be involved in cancer, HIV/AIDS, tuberculosis, malaria, or the toxic effects produced by exposure to industrial chlorinated aromatics or the like. Thus, the scaffold artificial receptor can treat these disorders.

The scaffold molecule can be any of the variety of known molecular scaffolds employed in combinatorial research. Suitable scaffold molecules include those illustrated in Scheme 6. The compounds illustrated in Scheme 6 are either commercially available or can be made by known methods. For example, compounds 1, 2, 4, and 5 are commercially available from Aldrich. Compound 3 can be prepared by the method of Pattarawarapan (2000) (Pattarawarapan, M and Burgess, K, "A Linker Scaffold to Present Dimers of Pharmacophores Prepared by Solid-Phase Synthesis", Angew. Chem. Int. Ed., 39, 4299-4301 (2000)). Compound 6 can be made in the o-NH_2 form (shown) by the method of Kimura (2001) (Kimura, M; Shiba, T; Yamazaki, M; Hanabusa, K; Shirai, H and Kobayashi, N, "Construction of Regulated Nanospace around a Porphyrin Core", J. Am. Chem. Soc., 123, 5636-5642 (2001)) and in the p-COOH (not shown) by the method of Jain (2000) (Jain, RK; Hamilton, AD (2000), "Protein Surface Recognition by Synthetic Receptors Based on a Tetraphenylporphyrin Scaffold", Org. Lett. 2, pp. 1721-1723). Compound 7 can be made in the -COOH form (shown) or in the -OH form (not shown) by the method of Hamuro (1997) (Hamuro, Y. et al., (Andrew Hamilton), "A Calixarene with four Peptide Loops: An Antibody Mimic for Recognition of Protein Surfaces", Angew. Chem. Int. Ed. Engl., 36, pp. 2680-2683). Compound 8 can be used with three functional groups in the -NH_2 form (shown), with four functional groups including both the -COOH and -NH_2 groups (as

shown), or as a dimer product with 6 -NH_2 functional groups (not shown). Each of these forms of compound 8 can be made by the method of Opatz (2001) (Opatz, T; Liskamp, RM (2001), "A Selectively Deprotectable Triazacyclophane Scaffold for the Construction of Artificial Receptors", Org. Lett., 3, pp. 3499-3502).

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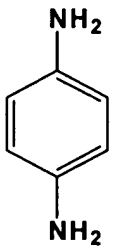

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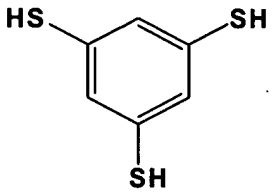

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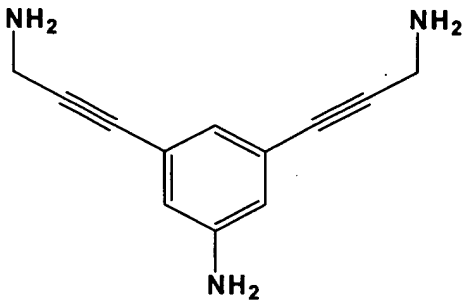

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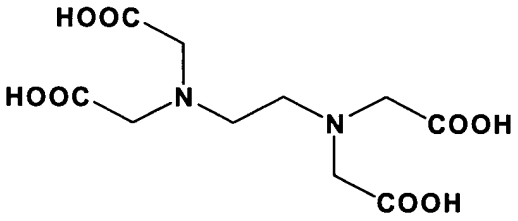

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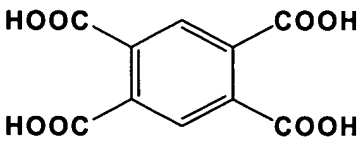

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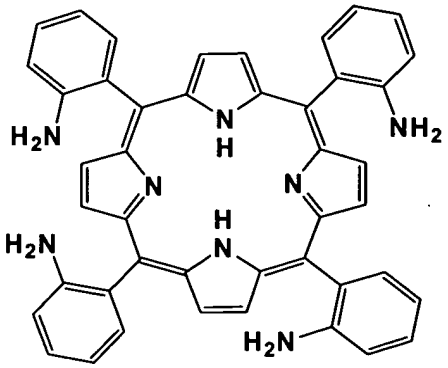

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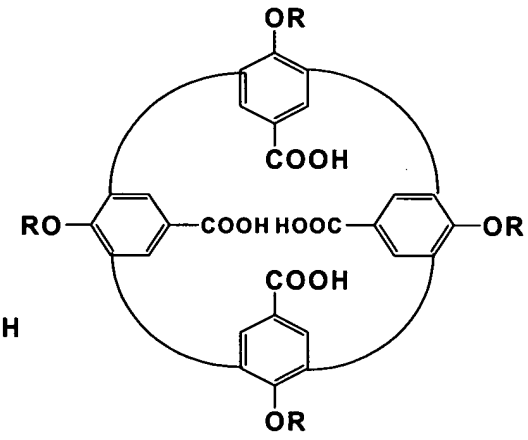

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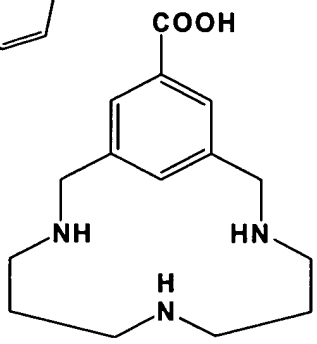

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Scheme 6

Molecular Configurations in Combinations of Building Blocks

Figure 20 schematically illustrates a molecular configuration of building blocks that can provide a region for binding for a small molecule ligand. Figure 20 illustrates that a plurality of adjacent building blocks, each with a pendant and an equatorial recognition element, can form a cavity or other binding site. The binding site can be sized to serve as a receptor for, for example, a small molecule ligand of interest. Space filling molecular models of embodiments of building blocks can be envisioned to fit this schematic. Neighboring building blocks that are different from one another can provide diversity to the binding interactions available in the binding site.

Figure 21 schematically illustrates a molecular configuration of building blocks that can provide a broad binding site with a large surface area. Figure 21 illustrates that a plurality of adjacent building blocks, each with two pendant lateral recognition elements, can form a broad binding site with a large molecular footprint. The broad binding site can serve as a receptor for, for example, a macromolecule ligand of interest, a cell, or a microorganism (e.g., a bacterium or a virus). Space filling molecular models of embodiments of building blocks can be envisioned to fit this schematic. Neighboring building blocks that are different from one another can provide diversity to the binding interactions available in the binding site.

Figure 22 schematically illustrates a molecular configuration of building blocks arranged to form a protruding binding site, which can, for example, bind a test ligand with a cavity. Figure 22 illustrates that a plurality of adjacent building blocks, each with a pendant protruding recognition element, can form a protruding binding site. The protruding binding site can serve as a receptor for, for example, a macromolecule having an active or binding site. Space filling molecular models of embodiments of building blocks can be envisioned to fit this schematic. Neighboring building blocks that are different from one another can provide diversity to the binding interactions available in the binding site. The binding site can include recognition elements from 2 or more building blocks.

Figure 12 illustrates that a molecular configuration of building blocks can form 6 positional isomers. This illustration places the building blocks at corners of a square, but the same is true of 4 vertices of any quadrilateral. Candidate or lead artificial receptors having the structure of the different positional isomers can be made on a scaffold.

Embodiments of Sets as Reagents

The present invention includes sets of building blocks as reagents. Reagent sets of building blocks can include individual or mixtures of building blocks. The reagent sets can be used to make immobilized building blocks and groups of building blocks, and can be sold for this purpose. In an embodiment, the set includes building blocks with recognition elements representing hydrophobic alkyl, hydrophobic aryl, hydrogen bond acceptor, basic, hydrogen bond donor, and small size as structural characteristics. For example, the set can include building blocks of general Formula 2, with RE_1 being B1, B2, B3, B3a, B4, B5, B6, B7, B8, or B9 and with RE_2 being A1, A2, A3, A3a, A4, A5, A6, A7, A8, or A9. In an embodiment of the set, RE_1 can be B1, B3, B3a, B6, or B8 and RE_2 can be A2, A4, A5, or A9. In an embodiment of the set, RE_1 can be B2, B4, or B6 and RE_2 can be A2, A4, or A6. In an embodiment of the set, RE_1 can be B2, B4, B6, or B8 and RE_2 can be A2, A4, A6, or A8. In an embodiment of the set, RE_1 can be B1, B2, B4, B6, or B8 and RE_2 can be A1, A2, A4, A6, or A8. In an embodiment of the kit, RE_1 can be B1, B2, B3, B3a, B4, B5, B6, B7, B8, or B9 and RE_2 can be A1, A2, A3, A3a, A4, A5, A6, A7, A8, or A9. In an embodiment of the kit, RE_1 can be B1, B2, B4, B6, B8, or B9 and RE_2 can be A1, A2, A4, A6, A8, or A9. The building blocks can include as L $(CH_2)_nCOOH$, with n=1-16, n=2-8, n=4-6, or n=3, or an activated form of L, for example, an activated ester.

The set can be part of a kit including containers of one or mixtures of building blocks, the containers can be in a package, and the kit can include written material describing the building blocks and providing instructions for their use.

Additional Embodiments of the Present Invention

The present artificial receptors can be prepared by methods including both focused combinatorial synthesis and targeted screening arrays. The present compositions and methods can combine the advantages of receptor focused synthesis and high throughput evaluation to rapidly identify and produce practical, target specific artificial receptors.

In an embodiment, the present invention includes a method of making a heterogeneous building block array. This method includes forming a plurality of spots on a

solid support, the spots including a plurality of building blocks, and coupling a plurality of building blocks to the solid support in the spots.

In an embodiment, the present invention includes a method of using an artificial receptor. This method includes contacting a heterogeneous building block array with a test ligand, detecting binding of a test ligand to one or more spots in the array, and selecting one or more of the binding spots as the artificial receptor. The artificial receptor can be a lead or working artificial receptor. The method can also include testing a plurality of building block arrays.

In an embodiment, the present invention includes a composition including a support with a portion of the support including a plurality of building blocks. The building blocks are coupled to the support. The composition can include or be an artificial receptor, a heterogeneous building block array, or a composition including a surface and a region on the surface.

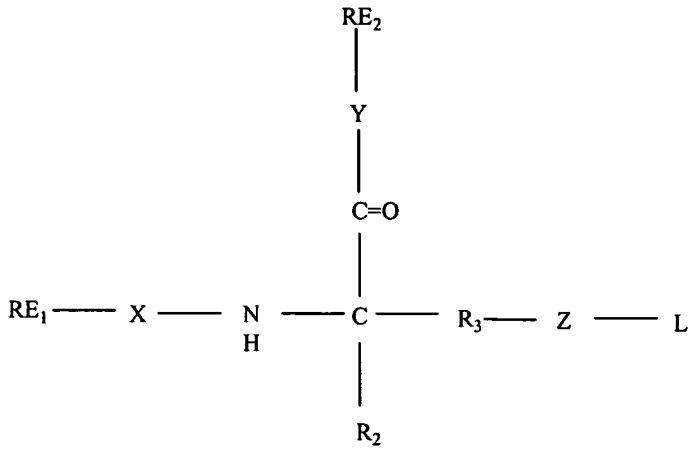
In an embodiment, the present invention includes an artificial receptor including a plurality of building blocks coupled to a support.

In an embodiment, the present invention includes a heterogeneous building block array. This array includes a support and a plurality of spots on the support. The spots include a plurality of building blocks. The building blocks are coupled to the support.

In an embodiment, the present invention includes a composition including a surface and a region on the surface. This region includes a plurality of building blocks, the building blocks being coupled to the support.

In an embodiment, the present invention includes a composition of matter including a plurality of building blocks.

In an embodiment, the building blocks include framework, linker, first recognition element, and second recognition element or have a formula linker-framework-(first recognition element)(second recognition element). The framework can be an amino acid. The building block can have the formula:



in which: X, Y, Z, R_2, R_3, RE_1, RE_2 and L are described hereinbelow.

The present invention may be better understood with reference to the following examples. These examples are intended to be representative of specific embodiments of the invention, and are not intended as limiting the scope of the invention.

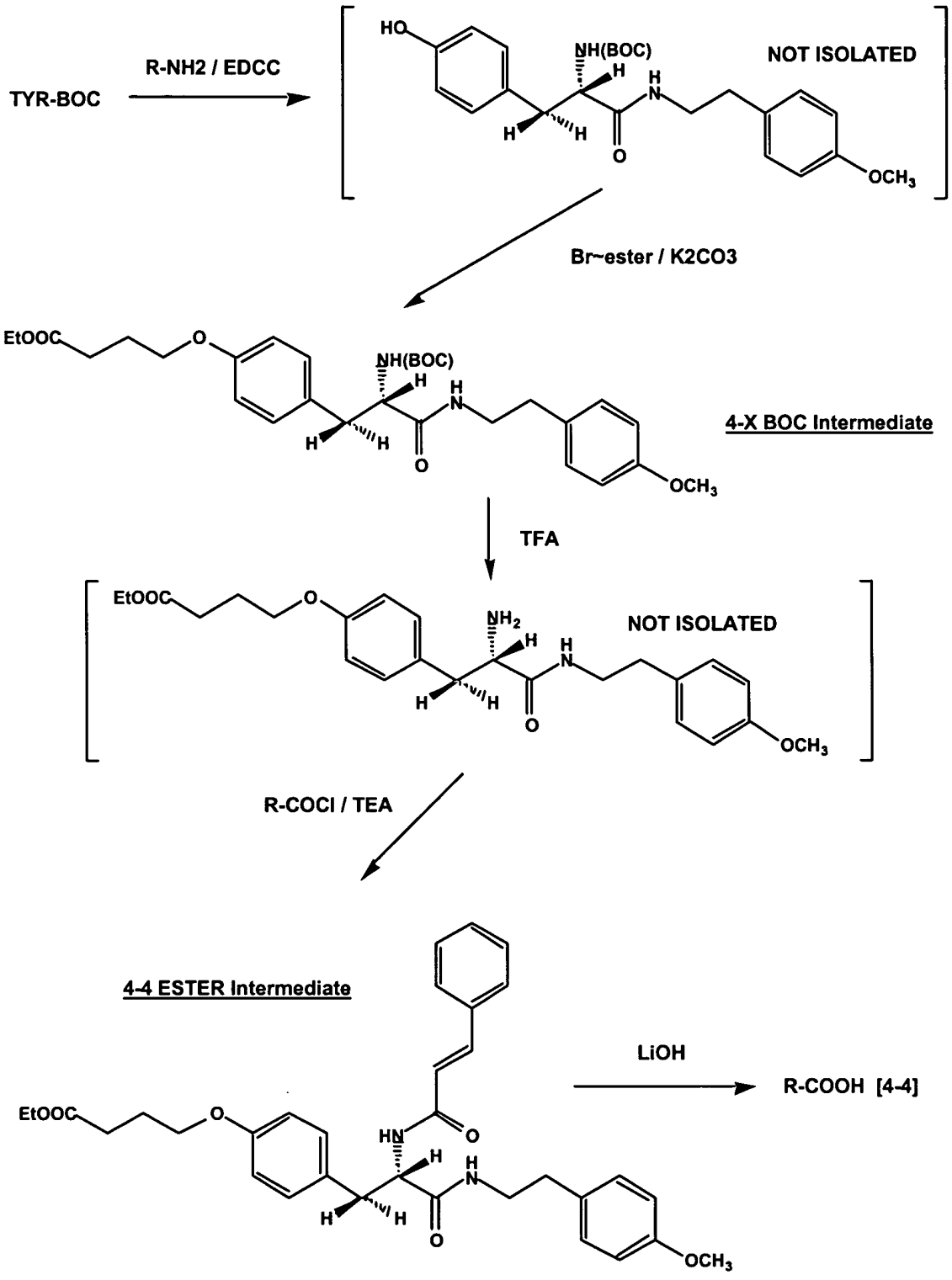
EXAMPLES

Example 1 - Synthesis of Building Blocks

Selected building blocks representative of the alkyl-aromatic-polar span of the an embodiment of the building blocks were synthesized and demonstrated effectiveness of these building blocks for making candidate artificial receptors. These building blocks were made on a framework that can be represented by tyrosine and included numerous recognition element pairs. These recognition element pairs were selected along the diagonal of Table 2, and include enough of the range from alkyl, to aromatic, to polar to represent a significant degree of the interactions and functional groups of the full set of 81 such building blocks.

Synthesis

Building block synthesis employed a general procedure outlined in Scheme 7, which specifically illustrates synthesis of a building block on a tyrosine framework with recognition element pair A4B4. This general procedure was employed for synthesis of building blocks including TyrA1B1 [1-1], TyrA2B2, TyrA2B4, TyrA2B6, TyrA2B8, TyrA4B2, TyrA4B4, TyrA4B6, TyrA4B8, TyrA6B2, TyrA6B4, TyrA6B6, TyrA6B8, TyrA8B2, TyrA8B4, TyrA8B6, TyrA8B8, and TyrA9B9, respectively.


TYR-BOC
R-NH2 / EDCC
HO
NH(BOC)
H
H
H
H
N
O
OCH3
NOT ISOLATED
Br~ester / K2CO3
EtOOC
O
NH(BOC)
4-X BOC Intermediate
TFA
NH2
NOT ISOLATED
R-COCl / TEA
4-4 ESTER Intermediate
LiOH
R-COOH [4-4]
HN

Scheme 7

Results

Synthesis of the desired building blocks proved to be generally straightforward. These syntheses illustrate the relative simplicity of preparing the building blocks with 2 recognition elements having different structural characteristics or structures (e.g. A4B2, A6B3, etc.) once the building blocks with corresponding recognition elements (e.g. A2B2, A4B4, etc) have been prepared via their X BOC intermediate.

The conversion of one of these building blocks to a building block with a lipophilic linker can be accomplished by reacting the activated building block with, for example, dodecyl amine.

Example 2 - Preparation and Evaluation of Microarrays of Candidate Artificial Receptors

Microarrays of candidate artificial receptors were made and evaluated for binding several protein ligands. The results obtained demonstrate the 1) the simplicity with which microarrays of candidate artificial receptors can be prepared, 2) binding affinity and binding pattern reproducibility, 3) significantly improved binding for building block heterogeneous receptor environments when compared to the respective homogeneous controls, and 4) ligand distinctive binding patterns (e.g., working receptor complexes).

Materials and Methods

Building blocks were synthesized and activated as described in Example 1. The building blocks employed in this example were TyrA1B1 [1-1], TyrA2B2, TyrA2B4, TyrA2B6, TyrA4B2, TyrA4B4, TyrA4B6, TyrA6B2, TyrA6B4, and TyrA6B6. The abbreviation for the building block including a linker, a tyrosine framework, and recognition elements AxBy is TyrAxBy.

Microarrays for the evaluation of the 130 n=2 and n=3, and for evaluation of the 273 n=2, n=3, and n=4, candidate receptor environments were prepared as follows by modifications of known methods. Briefly: Amine modified (amine "lawn"; SuperAmine Microarray plates) microarray plates were purchased from Telechem Inc., Sunnyvale, CA

(www.arrayit.com). These plates were manufactured specifically for microarray preparation and had a nominal amine load of 2-4 amines per square nm according to the manufacturer. The CAM microarrays were prepared using a pin microarray spotter instrument from Telechem Inc. (SpotBot™ Arrayer) typically with 200 um diameter spotting pins from Telechem Inc. (Stealth Micro Spotting Pins, SMP6) and 400-420 um spot spacing.

The 9 building blocks were activated in aqueous dimethylformamide (DMF) solution as described above. For preparing the 384-well feed plate, the activated building block solutions were diluted 10-fold with a solution of DMF/H_2O/PEG400 (90/10/10, v/v/v; PEG400 is polyethylene glycol nominal 400 FW, Aldrich Chemical Co., Milwaukee, WI). These stock solutions were aliquotted (10 μl per aliquot) into the wells of a 384-well microwell plate (Telechem Inc.). A separate series of controls were prepared by aliquotting 10 μl of building block with either 10 μl or 20 μl of the activated [1-1] solution. The plate was covered with aluminum foil and placed on the bed of a rotary shaker for 15 minutes at 1,000 RPM. This master plate was stored covered with aluminum foil at -20°C when not in use.

For preparing the 384-well SpotBot™ plate, a well-to-well transfer (e.g. A-1 to A-1, A-2 to A-2, etc.) from the feed plate to a second 384-well plate was performed using a 4 μl transfer pipette. This plate was stored tightly covered with aluminum foil at -20°C when not in use. The SpotBot™ was used to prepare up to 13 microarray plates per run using the 4 μl microwell plate. The SpotBot™ was programmed to spot from each microwell in quadruplicate. The wash station on the SpotBot™ used a wash solution of EtOH/H2O (20/80, v/v). This wash solution was also used to rinse the microarrays on completion of the SpotBot™ printing run. The plates were given a final rinse with deionized (DI) water, dried using a stream of compressed air, and stored at room temperature.

Certain of the microarrays were further modified by reacting the remaining amines with succinic anhydride to form a carboxylate lawn in place of the amine lawn.

The following test ligands and labels were used in these experiments:

1) r-Phycoerythrin, a commercially available and intrinsically fluorescent protein with a FW of 2,000,000.

2) Ovalbumin labeled with the Alexa™ fluorophore (Molecular Probes Inc., Eugene, OR).

3) BSA, bovine serum albumin, labeled with activated Rhodamine (Pierce Chemical, Rockford, IL) using the known activated carboxyl protocol. BSA has a FW of 68,000; the material used for this study had ca. 1.0 rhodamine per BSA.

4) Horseradish peroxidase (HRP) modified with extra amines and labeled as the acetamide derivative or with a 2,3,7,8-tetrachlorodibenzodixoin derivative were available through known methods. Fluorescence detection of these HRP conjugates was based on the Alexa 647-tyramide kit available from Molecular Probes, Eugene, OR.

5) Cholera toxin.

Microarray incubation and analysis was conducted as follows: For test ligand incubation with the microarrays, solutions (e.g. 500 μl) of the target proteins in PBS-T (PBS with 20 μl/L of Tween-20) at typical concentrations of 10, 1.0 and 0.1 μg/ml were placed onto the surface of a microarray and allowed to react for, e.g., 30 minutes. The microarray was rinsed with PBS-T and DI water and dried using a stream of compressed air.

The incubated microarray was scanned using an Axon Model 4200A Fluorescence Microarray Scanner (Axon Instruments, Union City, CA). The Axon scanner and its associated software produce a false color 16-bit image of the fluorescence intensity of the plate. This 16-bit data is integrated using the Axon software to give a Fluorescence Units value (range 0 - 65,536) for each spot on the microarray. This data is then exported into an Excel file (Microsoft) for further analysis including mean, standard deviation and coefficient of variation calculations.

Results

The CARA™: Combinatorial Artificial Receptor Array™ concept has been demonstrated using a microarray format. A CARA microarray based on N=9 building blocks was prepared and evaluated for binding to several protein and substituted protein ligands. This microarray included 144 candidate receptors (18 n=1 controls plus 6 blanks; 36 n=2 candidate receptors; 84 n=3 candidate receptors). This microarray demonstrated: 1) the simplicity of CARA microarray preparation, 2) binding affinity and binding pattern reproducibility, 3) significantly improved binding for building block heterogeneous receptor environments when compared to the respective homogeneous controls, and 4) ligand distinctive binding patterns.

Reading the Arrays

A typical false color/gray scale image of a microarray that was incubated with 2.0 μg/ml r-phycoerythrin is shown in Figure 23. This image illustrates that the processes of both preparing the microarray and probing it with a protein test ligand produced the expected range of binding as seen in the visual range of relative fluorescence from dark to bright spots.

The starting point in analysis of the data was to take the integrated fluorescence units data for the array of spots and normalize to the observed value for the [1-1] building block control. Subsequent analysis included mean, standard deviation and coefficient of variation calculations. Additionally, control values for homogeneous building blocks were obtained from the building block plus [1-1] data.

First Set of Experiments

The following protein ligands were evaluated for binding to the candidate artificial receptors in the microarray. The resulting Fluorescence Units versus candidate receptor environment data is presented in both a 2D format where the candidate receptors are placed along the X-axis and the Fluorescence Units are shown on the Y-axis and a 3D format where the Candidate Receptors are placed in an X-Y format and the Fluorescence Units are shown on the Z-axis. A key for the composition of each spot was developed (not shown). A key for the building blocks in each of the 2D and 3D representations of the results was also developed (not shown). The data presented are for 1-2 μg/ml protein concentrations.

Figures 24 and 25 illustrate binding data for r-phycoerythrin (intrinsic fluorescence). Figures 26 and 27 illustrate binding data for ovalbumin (commercially available with fluorescence label). Figures 28 and 29 illustrate binding data for bovine serum albumin (labeled with rhodamine). Figures 30 and 31 illustrate binding data for HRP-NH-Ac (fluorescent tyramide read-out). Figures 32 and 33 illustrate binding data for HRP-NH-TCDD (fluorescent tyramide read-out).

These results demonstrate not only the application of the CARA microarray to candidate artificial receptor evaluation but also a few of the many read-out methods (e.g. intrinsic fluorescence, fluorescently labeled, *in situ* fluorescence labeling) which can be utilized for high throughput candidate receptor evaluation.

The evaluation of candidate receptors benefits from reproducibility. The following results demonstrate that the present microarrays provided reproducible ligand binding.

The microarrays were printed with each combination of building blocks spotted in quadruplicate. Visual inspection of a direct plot (Figure 34) of the raw fluorescence data (from the run illustrated in Figure 23) for one block of binding data obtained for r-phycoerythrin demonstrates that the candidate receptor environment "spots" showed reproducible binding to the test ligand. Further analysis of the r-phycoerythrin data (Figure 23) led to only 9 out of 768 spots (1.2%) being deleted as outliers. Analysis of the r-phycoerythrin quadruplicate data for the entire array gives a mean standard deviation for each experimental quadruplicate set of 938 fluorescence units, with a mean coefficient of variation of 19.8%.

Although these values are acceptable, a more realistic comparison employed the standard deviation and coefficient of variation of the more strongly bound, more fluorescent receptors. The overall mean standard deviation unrealistically inflates the coefficient of variation for the weakly bound, less fluorescent receptors. The coefficient of variation for the 19 receptors with greater than 10,000 Fluorescent Units of bound target is 11.1%, which is well within the range required to produce meaningful binding data.

One goal of the CARA approach is the facile preparation of a significant number of candidate receptors through combinations of structurally simple building blocks. The following results establish that both the individual building blocks and combinations of building blocks have a significant, positive effect on test ligand binding.

The binding data illustrated in Figures 23-33 demonstrate that heterogeneous combinations of building blocks (n=2, n=3) are dramatically superior candidate receptors made from a single building block (n=1). For example, Figure 25 illustrate both the diversity of binding observed for n=2, n=3 candidate receptors with fluorescent units ranging from 0 to ca. 40,000. These data also illustrate and the ca. 10-fold improvement in binding affinity obtained upon going from the homogeneous (n=1) to heterogeneous (n=2, n=3) receptor environments.

The effect of heterogeneous building blocks is most easily observed by comparing selected n=3 receptor environments candidate receptors including 1 or 2 of those building blocks (their n=2 and n=1 subsets). Figures 35 and 36 illustrate this comparison for two

different n=3 receptor environments using the r-phycoerythrin data. In these examples, it is clear that progression from the homogeneous system (n=1) to the heterogeneous systems (n=2, n=3) produces significantly enhanced binding.

Although van der Waals interactions are an important part of molecular recognition, it is important to establish that the observed binding is not a simple case of hydrophobic/hydrophilic partitioning. That is, that the observed binding was the result of specific interactions between the individual building blocks and the target The simplest way to evaluate the effects of hydrophobicity and hydrophilicity is to compare building block logP value with observed binding. LogP is a known and accepted measure of lipophilicity, which can be measured or calculated by known methods for each of the building blocks. Figures 37 and 38 establish that the observed target binding, as measured by fluorescence units, is not directly proportional to building block logP. The plots in Figures 37 and 38 illustrate a non-linear relationship between binding (fluorescence units) and building block logP.

One advantage of the present methods and arrays is that the ability to screen large numbers of candidate receptor environments will lead to a combination of useful target affinities and to significant target binding diversity. High target affinity is useful for specific target binding, isolation, etc. while binding diversity can provide multiplexed target detection systems. This example employed a relatively small number of building blocks to produce ca. 120 binding environments. The following analysis of the present data clearly demonstrates that even a relatively small number of binding environments can produce diverse and useful artificial receptors.

The target binding experiments performed for this study used protein concentrations including 0.1 to 10 μg/ml. Considering the BSA data as representative, it is clear that some of the receptor environments readily bound 1.0 ug/ml BSA concentrations near the saturation values for fluorescence units (see, e.g., Figure 29). Based on these data and the formula weight of 68,000 for BSA, several of the receptor environments readily bind BSA at ca. 15 picomole/ml or 15 nanomolar concentrations. Additional experiments using lower concentrations of protein (data not shown) indicate that, even with a small selection of candidate receptor environments, femptomole/ml or picomolar detection limits have been attained.

One goal of artificial receptor development is the specific recognition of a particular target. Figure 39 compares the observed binding for r-phycoerythrin and BSA. Comparison of the overall binding pattern indicates some general similarities. However, comparison of specific features of binding for each receptor environment demonstrates that the two targets have distinctive recognition features as indicated by the (*) in Figure 39.

One goal of artificial receptor development is to develop receptors which can be used for the multiplexed detection of specific targets. Comparison of the r-phycoerythrin, BSA and ovalbumin data from this study (Figures 25, 27, 29) were used to select representative artificial receptors for each target. Figures 40, 41 and 42 employ data obtained in the present example to illustrate identification of each of these three targets by their distinctive binding patterns.

Conclusions

The optimum receptor for a particular target requires molecular recognition which is greater than the expected sum of the individual hydrophilic, hydrophobic, ionic, etc. interactions. Thus, the identification of an optimum (specific, sensitive) artificial receptor from the limited pool of candidate receptors explored in this prototype study, was not expected and not likely. Rather, the goal was to demonstrate that all of the key components of the CARA: Combinatorial Artificial Receptor Array concept could be assembled to form a functional receptor microarray. This goal has been successfully demonstrated.

This study has conclusively established that CARA microarrays can be readily prepared and that target binding to the candidate receptor environments can be used to identify artificial receptors and test ligands. In addition, these results demonstrate that there is significant binding enhancement for the building block heterogeneous (n=2, n=3, or n=4) candidate receptors when compared to their homogeneous (n=1) counterparts. When combined with the binding pattern recognition results and the demonstrated importance of both the heterogeneous receptor elements and heterogeneous building blocks, these results clearly demonstrate the significance of the CARA Candidate Artificial Receptor -> Lead Artificial Receptor -> Working Artificial Receptor strategy.

Example 3 - Preparation and Evaluation of Microarrays of Candidate Artificial Receptors Including Reversibly Immobilized Building Blocks

Microarrays of candidate artificial receptors including building blocks immobilized through van der Waals interactions were made and evaluated for binding of a protein ligand. The evaluation was conducted at several temperatures, above and below a phase transition temperature for the lawn (*vide infra*).

Materials and Methods

Building blocks 2-2, 2-4, 2-6, 4-2, 4-4, 4-6, 6-2, 6-4, 6-6 where prepared as described in Example 1. The C12 amide was prepared using the previously described carbodiimide activation of the carboxyl followed by addition of dodecylamine.

Amino lawn microarray plates (Telechem) were modified to produce the C18 lawn by reaction of stearoyl chloride (Aldrich Chemical Co.) in A) dimethylformamide / PEG 400 solution (90:10, v/v, PEG 400 is polyethylene glycol average MW 400 (Aldrich Chemical Co.) or B) methylene chloride / TEA solution (100 ml methylene chloride, 200 ul triethylamine) using the lawn modification procedures generally described in Example 2.

The C18 lawn plates where printed using the SpotBot standard procedure as described in Example 2. The building blocks were in printing solutions prepared by solution of ca. 10 mg of each building block in 300 ul of methylene chloride and 100 ul methanol. To this stock was added 900 ul of dimethylformamide and 100 ul of PEG 400. The 36 combinations of the 9 building blocks taken two at a time (N9:n2, 36 combinations) where prepared in a 384-well microwell plate which was then used in the SpotBot to print the microarray in quadruplicate. A random selection of the print positions contained only print solution.

The selected microarray was incubated with a 1.0 μg/ml solution of the probe protein (e.g. fluorescently labeled cholera toxin B) using the following variables: the microarray was washed with methylene chloride, ethanol and water to create a control plate, the microarray was incubated at 4 °C, 23 °C, or 44 °C. After incubation, the plate(s) were rinsed with water, dried and scanned (AXON 4100A). Data analysis was as described in Example 2.

Results

A control array from which the building blocks had been removed by washing with organic solvent did not bind cholera toxin (Figure 43). Figures 44-46 illustrate fluorescence signals from arrays printed identically, but incubated with cholera toxin at 4 °C, 23 °C, or 44 °C, respectively. Spots of fluorescence can be seen in each array, with very pronounced spots produced by incubation at 44 °C. The fluorescence values for the spots in each of these three arrays are shown in Figures 47-49. Fluorescence signal generally increases with temperature, with many nearly equally large signals observed after incubation at 44 °C. Linear increases with temperature can reflect expected improvements in binding with temperature. Nonlinear increases reflect rearrangement of the building blocks on the surface to achieve improved binding, which occurred above the phase transition for the lipid surface (*vide infra*).

Figure 50 can be compared to Figure 48. The fluorescence signals plotted in Figure 48 resulted from binding to reversibly immobilized building blocks on a support at 23 °C. The fluorescence signals plotted in Figure 50 resulted from binding to covalently immobilized building blocks on a support at 23 °C. These figures compare the same combinations of building blocks in the same relative positions, but immobilized in two different ways.

Figure 51 illustrates the changes in fluorescence signal from individual combinations of building blocks at 4 °C, 23 °C, or 44 °C. This graph illustrates that at least one combination of building blocks (candidate artificial receptor) exhibited a signal that remained constant as temperature increased. At least one candidate artificial receptor exhibited an approximately linear increase in signal as temperature increased. Such a linear increase indicates normal temperature effects on binding. The candidate artificial receptor with the lowest binding signal at 4 °C became one of the best binders at 44 °C. This indicates that rearrangement of the building blocks of this receptor above the phase transition for the lipophilic lawn produced increased binding. Other receptors characterized by greater changes in binding between 23 °C and 44 °C (compared to between 4 °C and 23 °C) also underwent dynamic affinity optimization.

Conclusions

This experiment demonstrated that an array including reversibly immobilized building blocks binds a protein substrate, like an array with covalently immobilized building blocks. The binding increased nonlinearly as temperature increased, indicating that movement of the building blocks increased binding. The candidate artificial receptors demonstrated improved binding upon mobilization of the building blocks.

It should be noted that, as used in this specification and the appended claims, the singular forms "a," "an," and "the" include plural referents unless the content clearly dictates otherwise. Thus, for example, reference to a composition containing "a compound" includes a mixture of two or more compounds. It should also be noted that the term "or" is generally employed in its sense including "and/or" unless the content clearly dictates otherwise.

It should also be noted that, as used in this specification and the appended claims, the phrase "adapted and configured" describes a system, apparatus, or other structure that is constructed or configured to perform a particular task or adopt a particular configuration to. The phrase "adapted and configured" can be used interchangeably with other similar phrases such as arranged and configured, constructed and arranged, adapted, constructed, manufactured and arranged, and the like.

All publications and patent applications in this specification are indicative of the level of ordinary skill in the art to which this invention pertains.

The invention has been described with reference to various specific and preferred embodiments and techniques. However, it should be understood that many variations and modifications may be made while remaining within the spirit and scope of the invention.